IN: Fish Physiology (Hoar, WS, DJ Randall, & J.R. Brett, eds.).

8

POPULATION GENETICS*

FRED W. ALLENDORF and FRED M. UTTER

I. INTRODUCTION

The title of this chapter covers a very broad area of study and is perhaps slightly misleading. A more exact description of the topic to be presented is the use of isozymic gene frequency data in the understanding of the genetic structure of fish populations. Almost all of our knowledge about the genetics of natural populations of fish has been gained from electrophoretic data—mostly within the present decade because of the maturation of isozyme methodology within this period.

* The authorship of this chapter is equal and alphabetical.

FISH PHYSIOLOGY, VOL. VIII

ISBN 0-12-350408-2

This recent increase of knowledge is part of a new era in the ease of studying genetic variation in natural populations (Lewontin, 1974). The genetic structure of most economically important fish populations still remains virtually unknown, however; even during the present decade hypothetical gene frequencies had to be postulated for populations of chinook salmon of the Columbia River because of the absence of gene frequency data (Simon, 1972) in spite of the fact that these populations have been the object of intensive fisheries research for many years.

A major area of fish population genetics which is not discussed in the present paper is that of quantitative genetics. For many years, domesticated fish populations have been subjected to massive artificial selection programs with the intent of producing more desirable fish. Until recently these programs have generally lacked an appreciation of the genetic processes underlying such changes in the genetic composition of populations. In recent years this situation has changed with two notable examples being quantitative genetic work with the rainbow trout (Gall, 1974, 1975) and the carp (Moav and Wohlfarth, 1973); it has become evident that the basic principles of quantitative genetics can be readily applied to the culture of fish. The reader interested in this area should examine these works as well as the following pertinent references (Aulstad *et al.*, 1972; Gjedrem, 1975, 1976; Sneed *et al.*, 1971; Cherfas, 1972).

Recognizing that major gaps remain in the understanding of population structures of fish, the methodology is now available for obtaining answers concerning the structure of fish populations that were previously impossible to obtain; a representative number of fish species has been studied electrophoretically for this reason. de Ligny (1969, 1972) reported that variants of an apparent genetic origin had been found in more than 47 species while reviewing electrophoretic studies carried out on fishes (predominantly teleosts) through 1970. Although much of the earlier work was primarily devoted to descriptions of genetic systems (presumably because of the novelty of the ease of detecting single gene variations in natural populations), some more penetrating biological questions were being asked concerning gene–environment interactions, physiological functions of variants, and population structures of species. A recently published book (Altukhov, 1974) discusses theoretical aspects of fish population genetics as well as presenting an extensive list of references of electrophoretic studies of fish populations. In a recent review article Utter *et al.* (1974) discuss what they regard as the most promising direction to be pursued through applications of electrophoretic data in the study of fishes. An extremely useful discussion of the applications of biochemical ge-

netics to aquaculture with emphasis on the lobster has also recently appeared (Hedgecock *et al.*, 1976).

Fish are a uniquely useful group of organisms for studies of population genetics. They are a large and highly variable group comprising nearly 45% of all vertebrate species. Kosswig (1973) has recently reviewed the role and advantages of using fish in research on genetics and evolution from the classical genetics perspective. Fish are especially useful for studies using isozymic techniques for a number of reasons. Fish populations, compared to those of other vertebrates, are generally quite large and collection of individuals is relatively easy. Fish occupy a wide range of aquatic (as well as some nonaquatic) habitats. Fish are poikilothermic animals and thus are more directly sensitive to environmental temperatures than are homeothermic vertebrates. Populations are structured by a variety of life history patterns including such extremes as anadromy—where a series of at least semi-isolated spawning populations spend the majority of their life cycle together in the ocean—to the other extreme of catadromy—where a single ocean spawning population diverges to spend the growing part of the life cycle in separate fish-water streams. Populations are also strongly influenced by geographical barriers to migration such as isolated lakes, streams, and impassable falls. Many fish species are easily cultured, permitting verification of Mendelian variants and experiments simulating environmental variables that may differentially affect particular allelic products. These are just some of the features that illustrate the usefulness of fish in studying evolutionary processes in natural populations.

Many fish populations are presently undergoing drastic fluctuations in size and distribution as a result of man's interest in their harvest and culture. Behnke (1973) has estimated that 99% of the original populations of cutthroat trout (*Salmo clarki*) in the interior of the United States have been lost in the last 100 years. Many other species of negligible direct importance to man, but of enormous importance indirectly through interactions with other species, have also been drastically affected by man's physical and biological manipulation of the environment. Understanding the present structure of fish populations therefore has a significant practical dimension superimposed on the pure scientific interest of these findings. The effects of plantings and the excessive harvest of existing populations can be best evaluated and managed only if the present population structures are known.

The present paper is intended to complement and extend the review of Utter *et al.* (1974). The emphasis is directed toward the use of isozymic genetic data in studies of fish populations with special focus

on the application of this data to the culture and management of fish populations. We have directed much of our discussion toward the salmonid fishes because of the importance of salmonids and our own extensive experience with this group, although the same principles are applicable to other organisms. A preliminary methodology section is presented which emphasizes the amount of genetic information available and the importance of confirming the genetic basis of electrophoretic variants. An overview of some of the areas where isozyme studies have been used in the study of fish populations is followed by a more detailed examination of the concept of the amount and distribution of genetic variation in populations of fish. The final section discusses the management applications of this population genetic data in both artificial and natural populations of fish.

II. ISOZYME METHODOLOGY

A. Basic Techniques of Electrophoresis

The major advantages of electrophoretic methodology for genetic studies of populations are (1) the relative ease of application and efficiency of the techniques and (2) the direct relationship between appropriately chosen protein variants and the gene. The basic electrophoretic methodology has been presented in detail elsewhere (see Utter *et al.*, 1974, for gel techniques; Shaw and Prasad, 1970; for staining methods). The present discussion is supplemental to these descriptions and emphasizes ways in which a greater amount of genetic information per individual can be obtained.

Optimal resolution for a given protein system involves a number of variables including buffer system, distance of migration in a given buffer system, tissue in which protein is expressed, and species. As an example, we will consider enzyme systems in rainbow trout that are affected by this kind of variation.

Malate dehydrogenase (MDH) of rainbow trout (*Salmo gairdneri*) occurs in two cytoplasmic forms and at least two mitochondrial forms (reviewed in Clayton *et al.*, 1975). The distribution of these forms in different tissues and their expression on different buffer systems is summarized in Table I. The alkaline buffer systems (1 and 2) resolve clearly only the B form of MDH in muscle extracts while the acidic buffer systems (3 and 4) have a much broader range of expression. Many other protein systems have optimal expression on the alkaline buffer systems and most of the genetic variation of MDH reported in

Table I

Tissue Distribution and Expression of MDH Isozymes of Rainbow Trout in Different Tissues with Different Buffer Systems

Buffer system[a]	Muscle MDH[b]			Liver MDH			Eye MDH		
	A	B	M	A	B	M	A	B	M
1 and 2	0	+++[c]	0	0	0	0	0	0	0
3 and 4	+	++	+	+++	+	0	+++	+	0

[a] (1) Tris–citrate gel buffer, pH 8.5; lithium hydroxide–boric acid tray buffer, pH 8.1 (Ridgway *et al.*, 1970). (2) Tris–boric acid–EDTA continuous system, pH 8.7 (Markert and Faulhaber, 1965). (3) Sodium phosphate, pH 6.5 (Wolf *et al.*, 1970). (4) *N*-(3-Aminopropyl)-morpholine-citrate, pH 6.1 (Clayton and Tretiak, 1972).

[b] Forms of MDH: (A) cytoplasmic A; (B) cytoplasmic B; (M) mitochondrial.

[c] +, ++, and +++ *indicate relative intensities with clear expression;* 0 *indicates* no activity or inadequate resolution.

rainbow trout occurs in the B system; thus, it may seem expedient not to screen for MDH activity on acidic buffer systems. However, genetic variants have been seen for the A and mitochondrial systems as well (Allendorf *et al.*, 1975; Clayton *et al.*, 1975) and data from these systems should be included in population surveys where possible.

Similar variation with buffer systems is seen with other enzymes. For instance, one locus is expressed on the alkaline systems and at least three loci are resolved with the acid systems for α-glycerophosphate dehydrogenase (AGPD) from muscle extracts of rainbow trout. Clear expression for rainbow trout AGPD variants in buffer system (1) is obtained only if marker dye migration has not exceeded 4 cm; AGPD variants in pink salmon are clearly resolved on all of the buffer systems independent of migration distance. Glucose-6-phosphate dehydrogenase (G6PD) expression from liver extracts of rainbow trout is clear only on buffer system (1) and then only if the total anodal migration has not exceeded 4 cm. These examples illustrate the kinds of variability of expression seen for many protein systems with regard to different buffer systems, migration distances, tissues, and species. It is necessary to effectively deal with these variables or risk the loss of genetic information.

Broadening the capability for specific staining of enzyme systems is also necessary for increasing the amount of genetic information that can be obtained. Fluorescent detection of enzyme activity is neither new nor inadequately described in the literature (Coates *et al.*, 1975; Chen *et al.*, 1972) but has not received the universal application of other staining methods. We have recently incorporated fluorescent techniques into our methodology and review them here because (1)

they expand the number of genetic systems that can be examined, and (2) they have the potential for giving greater sensitivity and economy to some staining methods.

We use fluorescent staining methods for two major categories of enzymes. A method is available for staining for specific esterases using esters of the fluorescent compound 4-methyl umbelliferone. This procedure has many advantages over the usual azo-dye linked methods. It is both more specific and sensitive which allows detection of loci not previously detectable. In addition, these fluorescent methods are quick, simple, reliable, and inexpensive.

The other enzyme category which can be examined using fluorescent methods is NAD and NADP dependent enzymes. These procedures take advantage of the fluorescence of the reduced cofactor at the site of enzyme activity. The staining procedure is the same as the conventional method except that a tetrazolium salt (either NBT or MTT) and PMS are not used. The advantages of the method are that it is less expensive and more sensitive than the conventional method. A disadvantage is that immediate recording of the data is necessary because of diffusion of the fluorescent product and the eventual loss of fluorescence.

The potential uses of fluorescent staining procedures are not limited to these applications. A variety of other fluorescent procedures are possible; the further exploitation of these precedures should play an important role in increasing the number of loci which can be examined by electrophoresis.

B. Data Interpretation

Electrophoretic variants are useful for genetic analysis of populations only if the data reliably reflect genetic variations. A serious potential pitfall of the inexperienced worker seeking genetic data from electrophoretic patterns is attributing a genetic basis to nongenetic variations. The genetic variants we are concerned with are a stable attribute of the individual and once expressed in the organism's development, remain qualitatively the same throughout the remainder of its life. Variations in protein expression from a given tissue reflecting other than simple Mendelian differences among individuals may arise from a number of causes including (1) ontogenetic changes in gene expression (Shaklee *et al.*, 1974), (2) changes reflecting environmental differences such as temperature, salinity, or disease (Hochachka and Somero, 1973; Amend and Smith, 1974), (3) changes

resulting from dissection or extraction procedures, and (4) changes brought about from conditions and length of storage. Nongenetic variation must be ruled out prior to concluding that a particular variant has a simple genetic basis; the use of such data in making genetic comparisons among populations can be grossly misleading and result in erroneous conclusions.

We have observed a considerable amount of electrophoretic variation of both enzymes and nonenzymatic proteins that lack a genetic basis. Two instances that were particularly deceiving were variations in liver esterases of sockeye salmon (*Oncorhynchus nerka*) and the sculpin (*Leptocottus armatus*). Initial extractions in both cases indicated two allele systems of monomeric enzymes with phenotypic frequencies fitting a Hardy–Weinberg distribution. Extractions made a day later from the same livers showed different patterns of variation (e.g., extracts from the same liver would frequently give single-banded phenotypes one day, and double-banded or the alternate single-banded phenotype on the other day and vice versa).

The ultimate test of the genetic basis of a particular variant is actual breeding data where the phenotypic ratios of progeny for a particular protein system are consistent with the known phenotypes of the parents. Family data are impractical or impossible to obtain for many organisms; however, other data are usually sufficient to give assurance that a particular pattern of variation has a genetic basis. Genetic variants are usually evident on the basis of predictable banding patterns of heterozygotes for a given protein system; heterozygotes of a monomer, dimer, and tetramer predictably give rise to one, three, and five-banded phenotypes, respectively (for more detailed discussions of relationships between subunit structures and heterozygous phenotypes, see Shaw, 1964; Utter *et al.*, 1974). The expression of a predictable pattern of electrophoretic variation for a given protein system in a species is usually sufficient evidence for simple genetic variation, provided consistent individual phenotypes are obtained from multiple tests of a tissue.

The strongest criterion, next to family data, for a genetic basis of a particular protein variant is parallel expression of the variant in different tissues of the organism. Such expression is virtual confirmation of a genetic polymorphism. A weakness of this criterion, if used alone, is that the converse situation, when a variant is expressed in only a single tissue, is not positive evidence for artifactual expression because of the possibility of the expression of specific genes in single tissues. This principle is demonstrated in the expression of aspartate aminotransferase (AAT) in muscle and eye extracts of salmonids (Allendorf,

1975; May, 1975; Allendorf and Utter, 1976). The common form in both tissues has identical electrophoretic mobility; but genetic variants have demonstrated that two loci code for muscle AAT while a third locus is expressed in the eye. In spite of this caution, parallel expression in different tissues remains strong positive evidence for genetic polymorphism.

The extensive gene duplication of salmonid fishes, and certain other teleosts having an apparent tetraploid ancestry (Ohno, 1970), complicate interpretations of electrophoretic patterns. Approximately 50% of the structural gene loci detected by electrophoretic methods are duplicated in rainbow trout and Pacific salmon (Allendorf *et al.*, 1975). Complexities of interpretation arise when subunits encoded by different loci form active proteins of similar or identical electrophoretic mobility. This situation is outlined in Fig. 1 for a dimeric protein where patterns of duplicated and nonduplicated loci are compared. Electrophoretic expression is based on four rather than two gene doses, and the number of possible phenotypes is five rather than three for a simple two allele polymorphism. Interpretation of the three phenotypes where both kinds of subunits are expressed becomes quantitative rather than qualitative and is based on the relative intensity of the three bands rather than the presence or absence of particular bands.

Two genetic possibilities exist for this kind of variation, disomic inheritance at two loci or a single tetrasomic locus. It has been shown that the distinction between two polymorphic disomic loci and a single polymorphic tetrasomic locus can be made only through breeding studies (Allendorf *et al.*, 1975). Only disomy has been demonstrated in salmonids where breeding data have been available (Allendorf and Utter, 1973; Allendorf *et al.*, 1975; May *et al.*, 1975).

Disomic inheritance of two polymorphic loci creates ambiguities regarding assignment of genotypes to phenotypes where both kinds of subunits are expressed. Phenotypes expressing 3 : 1 ratios of subunits are homozygous for one locus and heterozygous for the other, but the

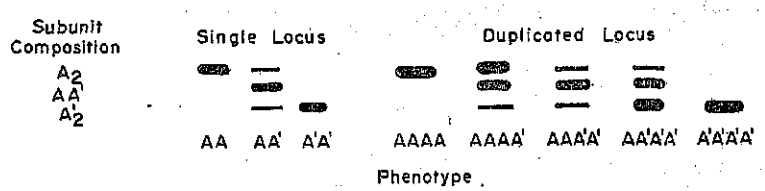

Fig. 1. Phenotypes expected from genetic variation of a dimeric protein expressed at a single and a duplicated locus (A = allele *100*; A' = allele *75*).

loci cannot be assigned. The phenotype expressing 2:2 ratios of subunits may be either heterozygous at both loci or homozygous at both loci for different alleles.

The variability of expression of the common phenotypes of the dimeric enzyme phosphoglucose isomerase (PGI) in different tissues of four salmonid species is useful in demonstrating some of the principles of identification of biochemical genetic variation in a well-studied and somewhat complex system. The molecular basis of PGI isozymes of salmonids was identified by Avise and Kitto (1973) through observation of monomorphic phenotypes and has been confirmed by subsequent observations and breeding studies involving genetic variants of PGI (May, 1975). PGI phenotypes from eye, muscle, heart, and liver of rainbow trout, cutthroat trout, coho salmon (*Oncorhynchus kisutch*), and masu salmon (*O. masou*) (Fig. 2) display a potentially confusing array of patterns. Three loci code for PGI activity in these species, *PGI-1*, *PGI-2*, and *PGI-3*, listed in order of increasing mobility of the common homodimers. PGI subunits appear to unite at random in these species within and between loci, giving rise to six-banded common phenotypes from extracts of skeletal muscle, a tissue in which each locus is expressed at approximately the same intensity.

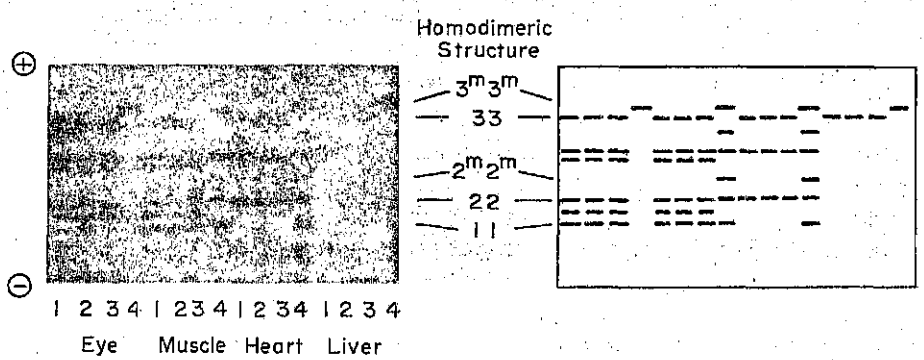

Fig. 2. Expression of PGI from four tissues of salmonids. (1) Rainbow trout; (2) cutthroat trout; (3) coho salmon; (4) masu salmon. Subunits for *PGI-1* produce electrophoretically identical homodimers in each of these species and are indicated by 1. Subunits for *PGI-2* and *PGI-3* in the two trout species and in coho salmon produce electrophoretically identical homodimers and are indicated by 2 and 3; subunits for *PGI-2* and *PGI-3* in masu salmon produce more negatively charged homodimers than the other species and are indicated by 2^m and 3^m. The other bands seen are heterodimers formed by the combination of protein subunits from two different loci. These heterodimers are intermediate in mobility between the homodimers of the two subunits present in the heterodimer.

Expression of the three PGI loci varies in the other tissues. *PGI-3* is strongly expressed in all tissues of all species; in the liver it is the only locus giving rise to clearly distinguishable bands. *PGI-1* and *PGI-2* are expressed at reduced intensities compared to *PGI-3* in the eye; expression of both loci is virtually absent in the eye of the masu salmon. *PGI-2* and *PGI-3* are preferentially expressed in the heart except in the masu salmon where, as the skeletal muscle, all three loci are expressed.

There are some bands of Fig. 2 that are not explained by the above model, for example, a band slightly slower than the 33 band of rainbow trout (quite diffuse in tissues other than the eye), and a pair of bands between the 22 and the 33 bands that are particularly evident in the muscle of coho salmon. Such bands are most likely the result of posttranslational protein changes affecting the charge or shape of the molecule. We have observed a direct relationship between the occurrence of such additional PGI bands and the duration of frozen storage.

Genetic variation of PGI superimposes additional complexities beyond those observed in the homozygous phenotypes of Fig. 2. Additional bands are formed by random interactions of subunits both within and between loci if the variant allele codes for a subunit that is differently charged from any of those involved in the invariant phenotypes.

Often, however, a variant allele of one PGI locus codes for subunits giving rise to enzymes that are electrophoretically identical to the common forms of another locus; we have seen this kind of variation between *PGI-1* and *PGI-2* in a number of salmonid species. Muscle extracts of heterozygous individuals give rise to six-banded phenotypes but the bands have uneven intensities because of the disproportionate synthesis of subunits. The eye PGI phenotype of coho salmon in Fig. 2 is similar to a heterozygous phenotype in which a *PGI-1* variant allele gives rise to a subunit which is electrophoretically identical to the common form of *PGI-2*. However, the even intensity of the bands of the muscle phenotype of coho salmon indicates that uneven intensities of the bands containing the *PGI-1* and the *PGI-2* subunits in the eye reflects differential synthesis of subunits of the two loci in the eye of this individual rather than allelic variation.

The complexity of PGI expression in salmonids is matched (or surpassed) by other systems such as lactate dehydrogenase (LDH) and MDH. A competent worker must acquire an understanding of the genetic significance of qualitative and quantitative variations observed in any complex system if data from that system are to be used in the study of populations.

Null alleles (i.e., alleles which either do not produce a protein or produce a protein with greatly reduced activity) are a phenomenon that can complicate interpretations of electrophoretic data. They are difficult to detect in simple systems (where only a single locus is expressed) except through the absence of activity in the homozygote. Detection becomes even more difficult where multiple loci are expressed. It can be impossible to differentiate between a null allele and an allele giving rise to an active protein having the same mobility as a protein for the same enzyme encoded by another locus. Dosage of heterozygotes in these instances is not a reliable criterion because it is difficult to differentiate between an active heterozygote [where a 3:1 ratio of subunits interact (see Fig. 1)] and a null heterozygote (with a 2:1 ratio of subunits). Null alleles must be considered in isozyme studies because of the tendency to identify heterozygotes of the null allele as a homozygote for the active allele which is present. This confusion results in distorted estimates of genotype frequencies and a deficiency of heterozygotes observed.

Before moving on we would like to emphasize the importance of both the quality and the quantity of the electrophoretic data collected in the study of populations. Meaningful parameters pertaining to the genetic variability and the identity of a particular taxon can only be estimated with data from many loci. It has been observed that estimates of average heterozygosity in a population obtained from data from 20 to 25 loci are highly correlated with estimates of variability that were derived from morphological criteria (Soule *et al.*, 1973); we regard this number of loci as a minimum for obtaining reliable estimates for average heterozygosity (see Section IV,B). Avise (1974) has emphasized the need for examining large numbers of loci in attempts to derive systematic relationships among conspecific populations (see also Sections III,B and V,B).

Amid the need to collect data from as many loci as possible, it is important to exercise restraint in the distinction of genetic polymorphism and artifact. If interpretation of a particular system is doubtful because of inadequate resolution or inexplicable patterns, it is better to disregard that system rather than to make guesses in the absence of reasonable assurance. If multiple bands are observed in the absence of genetic variation, it is best to conservatively estimate the number of loci involved, and it is almost certainly erroneous to ascribe a separate locus to each band. It is much easier to modify a conservative interpretation later as more data become available than to defend or retract an erroneous conclusion drawn from inappropriately interpreted data.

C. A Uniform System of Nomenclature

Many systems of nomenclature have arisen to describe allelic variation that is detected by electrophoresis. Most of these systems are based either on electrophoretic mobility or frequency of variants. Symbols describing allelic variants (often presented as superscripts following a designated locus) include letters, numbers, and different numbers of apostrophes. Use of one or another system of nomenclature has been rather arbitrary in electrophoretic studies of fishes and has often been based on the personal preferences of the worker who first described a particular electrophoretic variant.

Most systems of nomenclature are unambiguous if only a single locus and two alleles are expressed for a particular biochemical system. The potential for confusion rises rapidly, however, as the number of loci and alleles increase. Wright *et al.* (1975) have summarized a particularly confusing set of systems of nomenclature that have been used by various investigators for five loci coding for LDH in salmonids. This potential for confusion is detrimental to effective communication, and we propose here a uniform system of nomenclature that minimizes ambiguity.

The system that we propose has been used by investigators of *Drosophila* for some time (e.g., Prakash *et al.*, 1969). An abbreviation is chosen to designate each protein; when in italics, these same abbreviations represent the loci coding for these proteins. In the case of multiple forms of the same enzyme, a hyphenated numeral is included; the form with the least anodal migration is designated one, the next two, and so on. Allelic variants are designated according to the relative electrophoretic mobility. One allele (generally the most common one) is arbitrarily designated *100*. This unit distance represents the migration distance of the isozyme coded for by this allele. Other alleles are then assigned a numerical value representing their mobility relative to this unit distance. Thus, an allele of the most cathodal LDH locus coding for an enzyme migrating one-half as far as the common allele would be designated *LDH-1*(*50*).

III. MAJOR AREAS OF CURRENT INVESTIGATIONS

The application of the techniques of electrophoresis to the study of fish populations has found a broad base of biological questions of both basic and applied interest. The following section outlines the major categories of these investigations. This discussion is intended both to

point out the type of biological questions that can be pursued with fish as an organism and also to raise questions of special importance to the study of fish populations.

A. The Measurement of Natural Selection

The argument raging around the adaptive significance of isozymic polymorphisms has dominated the experimental population genetics literature in recent years. The controversy between the two conflicting groups, as colorfully portrayed by Lewontin (1974), has not subsided. Rather, it appears that polarization is increasing among strong advocates favoring either a selective or a neutral explanation for most of the genetic variation that has been detected by electrophoresis. Much of the most valuable experimental evidence towards resolving this controversy has come from studies of fish populations.

The most ambitious attempt to measure natural selection in natural populations of fish is that undertaken on the eelpout (*Zoarces viviparus*) in the seas surrounding Denmark. This fish is exceptionally well suited for this purpose because of its abundance, ease of capture, and its ovoviviparous life history. The young are large enough when taken from pregnant females to be typed electrophoretically for a series of enzymes which allows the verification of the genetic basis of the variation, including linkages (Hjorth, 1971). More importantly, a statistical procedure has been developed that uses such mother–offspring combinations to provide a much more powerful test for natural selection than is generally possible (Christiansen and Frydenberg, 1973; Christiansen *et al.*, 1973).

A very important result of this investigation is the demonstration of just how difficult it is to measure natural selection. The authors have carried out an intensive study of a single polymorphic esterase locus. In their procedure of partitioning selection into five components, they have calculated that the smallest selection differences that could have been detected with a 50% probability in these five components from a sample of 1282 adult and 782 offspring ranged from 7 to 33%. Since quite low selection differentials on the order of 1% is compatible with polymorphism at a large number of loci within the genome, it can be seen that the task of demonstrating such selection is formidable.

A more extensive report of this study involving the examination of 4206 adult and 2210 fetal young eelpouts has since appeared (Christiansen *et al.*, 1974). The only selection component that deviated significantly from the null hypothesis was that of zygotic selection acting in favor of both homozygotes at the expense of the heterozy-

gotes. This study does provide evidence of natural selection operating within a natural population for a specific isozyme locus. However, the demonstration of selection against the heterozygote does not provide the answer to the important question of how the polymorphism is maintained.

Systematic patterns of geographical variation in natural populations are often interpreted in adaptive terms. However, any geographical pattern of gene frequencies for a single locus can be explained by the random drift of neutral alleles if one is free to construct a suitable breeding structure. Even gene frequency clines can be explained by a model of neutrality (Kimura and Maruyama, 1971). Further evidence in the form of correlations between patterns of genotypic variation and specific characteristics of the environment is necessary. However, temperature, precipitation, and other environmental variables often are so strongly correlated with latitude, longitude, or altitude that it is difficult to relate particular patterns of genetic variation with environmental factors independent of geographical effects (Selander and Johnson, 1973). It is only when specific functional properties of allelic proteins can be related to the distribution of environmental variables that an adaptive interpretation becomes compelling.

A very fruitful area for such studies has been the examination of functional properties of fish isozymes in relation to temperature and their geographical distribution in populations of fish. Temperature, as an environmental variable, has the advantages of being both easy to measure in nature and having a relatively straightforward and determinable effect on enzyme kinetics. Some of the most convincing examples of the adaptive significance of allelic proteins have come from studies along these lines.

Merritt (1972) has demonstrated a functional basis for the maintenance of a cline for a two allele LDH polymorphism in the minnow *Pimephales promelas*. He has found that the homozygous form for the allele that predominates in northern areas possesses significantly higher Michaelis constants (K_m's) for pyruvate at 25°C and above than the isozymes of the other two genotypes. Thus, the pattern of variation of the northern allele in nature fits the distribution expected from *in vitro* studies. Koehn (1969) has also presented evidence of selective maintenance of esterase polymorphisms in two species of fish by finding associations between the temperature-dependent properties of the isozymes *in vitro* and their distribution in natural populations. Further evidence of temperature-dependent properties of isozymes and their distribution in nature was provided by Johnson (1971) in the

high cockscomb, *Anoplarchus purpurescens*. He demonstrated differential survival of larval LDH types at high and low temperatures through laboratory experiments. These experiments and field data showed a consistent correlation of allele frequencies with temperature supporting the author's conclusion of an adaptive interpretation of the polymorphism.

We would like at this time to interject a warning concerning a potential confounding factor in studies correlating *in vitro* enzymatic properties with geographical distribution. Electrophoresis is estimated to detect 33% of the possible amino acid substitutions (Lewontin, 1974); this allows a great deal of room for "hidden" genetic variation to exist. Such variation can sometimes cause a serious experimental problem. For example, assume a clinal polymorphism for which the allele frequencies are significantly correlated with temperature. To test our hypothesis of temperature being an important selective factor we propose to isolate the different allelic isozymes and to measure the effect of temperature on their kinetic properties. This isolation procedure is most easily accomplished by using the homozygotes for the particular allele. In doing this, however, there would be a tendency for one allelic product to come from fish from one end of the cline and the other allelic product from the other end of the cline. If we then detect kinetic differences in the isozymes derived in this manner, such differences could actually be due to some hidden allelic variation and not to the allelic differences we can measure with electrophoresis. The clinal distribution of the electrophoretic alleles could possibly be strictly due to geographically determined genetic drift.

Such an example is not totally hypothetical. Guilbert (cited in Utter *et al.*, 1974) studied K_m's of electrophoretically different forms of a two allele LDH system in sockeye salmon. He observed differences among the three phenotypes taken from fish returning to western Alaska, indicating that the heterozygous phenotypes had the greatest NADH binding capacity (i.e., lowest K_m) at 15°C. The common electrophoretic phenotype of western Alaskan fish was the only LDH form observed among sockeye returning to British Columbia and Washington. The K_m's of this phenotype were very different between the two areas, however. The K_m of Washington fish was the same as that of the heterozygous phenotype from western Alaska while the K_m of the common phenotype from western Alaska was much higher. The data suggest that the electrophoretically identical phenotype from two areas represents genetically different enzymes, and that conclusions relating function and distribution must be drawn with caution if data for different genetic types are collected from different areas.

An important present and future area of investigation is the examination of the effect of warm-water effluent on fish populations. The addition of warm-water effluent into the aquatic environment provides a situation where a possible genetic response of fish populations to this environmental change can be studied. Recent reports have examined this situation in three fish species (Mitton and Koehn, 1975; Nyman, 1975; Yardley *et al.*, 1974). Such investigations provide a "natural" experiment whereby possible natural selection on these loci can be sought. In addition, these studies have important practical implications as to possible harmful effects of warm-water effluent on populations of aquatic species.

Another very different approach to detecting natural selection in populations involves taking advantage of unusual life history patterns present in some species. The eel and salmon are fish with strikingly contrasting life histories. These extreme patterns have been used in the American eel and pink salmon to test hypotheses of selection versus neutrality at isozyme loci.

Investigations in the early decades of this century (Schmidt, 1922, 1925) provided evidence for a single mass spawning population of the American eel (*Anguilla rostrata*) in a region northeast of the West Indies. Eels from streams along the east coast of the United States were examined for gene frequencies of several isozyme loci under the assumption that any significant difference in gene frequencies between groups must be due to divergence from a common pool of zygotes (Williams *et al.*, 1973). Significant differences in gene frequencies were found both between groups of eels from different streams and between eels of different ages from the same streams. The authors interpreted this difference as strong evidence for a selective basis of the variation with the simple statement: "The alternative of selective neutrality can be immediately dismissed" (Williams *et al.*, 1973, p. 200).

We feel, however, that such conclusions must be based on a firmer understanding of the population structure of this species. The case for a single mass spawning panmictic population is not conclusive. Another problem that must be considered is the theoretical expectations of the result of this unusual life history where a single panmictic population disperses over such a large range of environments. It seems at least intuitively reasonable that large blocks of coadaptive genes are likely to evolve in such a situation. The effect then measured at a single locus may be the cumulative effect of a large number of genes in nonrandom association rather than a direct effect of the specific locus we are looking at. A more detailed experimental examination of

these populations should provide valuable evidence of the evolutionary processes which are actually present.

A study with pink salmon (*O. gorbuscha*) (Aspinwall, 1974) provides a highly interesting contrast to the eel study. Pink salmon mature at exactly 2 years of age (±10 days) and return to their native streams to spawn after spending the great majority of their life cycle in marine waters. The effect of this rigid timing is that there are two genetically isolated populations within many streams in odd and in even years. Confirmation of this rigid 2-year life cycle is the existence in the southern part of the range of large numbers of pink salmon returning in odd years but a complete absence of any even-year populations.

A number of populations from both even and odd years were examined for two polymorphic enzyme systems throughout the range of the pink salmon. The allele frequencies of the two systems revealed uniformity throughout the geographical range within even- or odd-year cycles. However, significant differences were found among some even- and odd-year populations that were sharing a single stream. These populations come as close as possible to sharing the same environment but yet being genetically isolated.

These results thus show uniformity of allele frequencies to even- or odd-year populations inhabitating a wide range of environmental situations, presumably due to a small amount of gene flow between populations of a given cycle. At the same time some populations sharing the same environment do not have similar allele frequencies, as would be expected under a model of selective maintenance of the polymorphisms. The author concludes that a model of selective neutrality combined with random genetic drift is the most plausible explanation of these results.

There are problems, however, in the assumption that similar environments will produce similar gene frequencies. A basic problem in this case is Lewontin's "historicity principle" (Lewontin, 1967). Lewontin shows that populations exposed to identical fluctuating environments, which differ only in the order of the environments, may exhibit vastly different gene frequencies. Thus, even if all biological assumptions are correct concerning these pink salmon populations, random environmental fluctuations from year to year may bring about *selected* gene frequency differences between odd- and even-year populations in the same stream.

These two studies provide striking contrast in both organisms studied and in conclusions drawn. The conclusions of both studies are somewhat preliminary in that they are based on important assump-

tions of the life cycles involved. Further results from these studies should provide more definite answers to the question being asked. Both of these studies are excellent examples of the kind of well-designed investigations that are needed to answer the current questions of population genetics. The differences point to the danger of extrapolating significantly beyond the experimental organism in question. Further studies simply reporting on genetic variation in additional species are not particularly useful at this time.

The study of the selective components of enzyme polymorphisms has important implications in the management of fish populations as well as being an important area of basic research. When using isozymic allele frequencies to delineate the boundaries of breeding populations we must have some assurance that we are dealing with relatively stable gene frequencies which are not being greatly changed by selection during the length of a generation. Such stability has been observed in salmonid populations that we have studied, as indicated in Section V.

In addition, there has been a widespread effort to detect the selective basis for particular polymorphisms and to turn this knowledge into practical use by artificial selection of a particular genotype. As stated before, a very low selection differential is compatible with the selective maintenance of polymorphisms. The hope of finding a large effect on economic performance of the vast majority of protein variants appears to be largely illusory (Robertson, 1972). This rather pessimistic prospect does not imply that these protein variants are without considerable potential for practical applications, however. We believe that the use of such allelic variants as effectively neutral markers of the genome is an extremely promising and relatively unexplored area of application. These applications are discussed in detail in Section V.

B. Systematics

Protein markers have found increasing use in systematic studies in recent years. Cumulative comparisons among loci between two taxonomic groups can be summarized by a variety of methods into indices of similarity or, conversely, genetic distance. Such data can be arranged in matrix form for comparisons among many taxonomic units, and dendrograms suggesting taxonomic relationships can be constructed from these matrices.

Avise (1974) has reviewed systematic studies using electrophoretic data and has concluded that electrophoretic techniques are an ex-

tremely valuable tool for systematics, particularly at the subgeneric level. The data are objective and have a purely genetic basis; a survey of the literature revealed that relationships indicated by electrophoretic data usually corresponded closely to those previously indicated by classical systematic criteria.

Ayala (1975) has compiled electrophoretic data from systematic studies of a broad range of organisms. His data reveal that similarity indices (based on Nei, 1972) in all organisms reported tend to average above 0.90 for populations within a species. The indices in *Drosophila* drop to about 0.80 for semi-species and sibling species, and considerably lower for morphologically distinct species. A similar reduction in genetic similarity as taxonomic distance increased was observed for other organisms.

Isolation of sympatric populations of chars and brown trout in certain Scandinavian lakes was suspected on the basis of different spawning times and locations, and differential growth rates; this genetic isolation was confirmed through electrophoretic studies (Nyman, 1972; Allendorf *et al.*, 1976). The reasons for this isolation of sympatric conspecific populations are unclear and may reflect recent convergence following previous isolation, or perhaps even sympatric speciation. Regardless of cause, it is clear that electrophoretic data are a powerful tool for studying the question of genetic isolation of sympatric populations.

Some electrophoretic studies dealing with systematic relationships among fish species are consistent with the tendency of electrophoretic data to confirm relationships established by other criteria, and the indication that the process of speciation involves considerable genetic reorganization. Studies of Pacific salmon (Utter *et al.*, 1973), sunfish (Avise and Smith, 1974), and rockfish (Johnson *et al.*, 1972) all fall into this category.

There are a number of interesting exceptions to these generalities among electrophoretic studies of fish systematics, however. Some of these studies concern comparisons among populations that have been given different specific, or even generic rank, but whose genetic similarities lie within the range usually attributed to conspecific populations. The specific status of some of the populations examined remains in question in spite of considerable morphological differentiation because of the allopatric nature of the populations and the fertility of hybrids between them. Such is the case with certain pupfish (*Cyprinodon*) populations of the southwest United States (Turner, 1974) and rainbow, golden (*Salmo aguabonita*), and red-banded trout (*Salmo* sp.) populations discussed in Section V of this review. High

genetic similarities among morphologically distinct pairs of fish species that occur sympatrically have been reported for rainbow and cutthroat trout (Utter *et al.*, 1973), two native cyprinids of California of different genera—hitch (*Lavinia*) and roach (*Hesperoleucus*) (Avise *et al.*, 1975), and two endemic cichlids of Mexico (Kornfield and Koehn, 1975). Such high genetic similarities among morphologically distinct and, in some instances, reproductively isolated populations are inconsistent with the much larger differences that are observed among most other species pairs and seem to be the result of recent speciation rather than a reflection of such factors as sampling error or convergent evolution (see Avise *et al.*, 1975, for a more detailed discussion of alternative possibilities). These studies indicate that the amount of genetic rearrangement involved in the process of speciation can vary considerably.

The above studies also suggest that loci determining metabolic proteins evolve at different rates in some organisms than those determining morphological characteristics. This observation is not limited to fishes. King and Wilson (1975) observed that man and chimpanzee are biochemically as closely related as subspecies of most other vertebrates studied. Cavalli-Sforza (1974) compared evolutionary trees of major human groups based respectively on purely genetic (i.e., protein and blood group) and anthropometric data and noted significant discrepancies between the two trees. He concluded that single gene data are probably more reliable for such reconstructions than are morphological data because of the limitations of an unknown number of gene loci and short-term environmental effects inherent in the latter method. This conclusion reinforces the potential value of electrophoretic data as a tool for systematic studies of fishes.

IV. GENETIC VARIATION IN POPULATIONS OF FISH

A. The Nature of Genetic Variation

Genetic variation among organisms sharing a common gene pool is necessary before evolutionary change can occur. The importance of genetic variation has long been recognized in the fields of plant and animal breeding. Knowledge of the amount of genetic variation present for a particular trait allows one to predict the amount of change expected from selection on that trait. If all of the loci relevant to the trait lack genetic variation, then no scheme of selection can be success-

ful. The measure of genetic variation important in this situation is defined as the heritability (h^2) of a trait and is expressed as

$$h^2 = \frac{V_A}{V_P}$$

where V_P is the phenotypic variance of the character in the population and V_A is the additive genetic variance of the trait (Falconer, 1960).

The amount of change expected under selection is a function of both the heritability of the trait under selection and the intensity of selection. It should be pointed out that since h^2 is a function of the amount of genetic variation in a population, the value of h^2 will undergo change in the course of a population's evolution. Therefore, this quantity is inadequate for predicting both the long-term progress expected under selection and the eventual limit to the selection process.

The widespread success of selection for traits in agricultural animals (Falconer, 1960; Brewbaker, 1964) is evidence of extensive genetic variation for many traits in a wide range of organisms. Even more impressive are the results of selection experiments with populations of *Drosophila*. There appears to be practically no character that cannot be successfully selected for in *Drosophila* (Lewontin, 1974). The conclusion from these results is that genetic variation exists in natural populations which is relevant to almost every aspect of an organisms's phenotype.

The description of genetic variation in populations is the fundamental observational basis of evolutionary genetics. There has historically been a great deal of controversy as to just how much genetic variation actually exists in natural populations (Lewontin, 1974). This controversy went unresolved in spite of the evidence from artifical selection experiments of a large amount of genetic variation in populations. It was not until the advent of electrophoresis, which has allowed the direct measuring of the genetic variation in natural populations at many loci, that this controversy could be settled. The presence of a high amount of genetic variability in a wide range of organisms has been convincingly demonstrated by isozyme studies over the last 10 years. These studies have been recently reviewed by Powell (1975).

B. Measuring Genetic Variation

It should be pointed out before detailing the measure of genetic variation in fish populations that the amount of genetic variation esti-

mated via electrophoresis is a minimum estimate. There is a large class of amino acid substitutions which is not detectable by electrophoresis because of the dependence of electrophoretic separation on changes in charge or shape of the protein molecule. It has been generally assumed that electrophoresis can detect approximately one-third of all possible amino acid substitutions. However, a detailed study of amino acid sequences in primate hemoglobin suggests that electrophoresis may in fact detect considerably less than one-third of the actual genetic variation present for a particular molecule (Boyer, 1972). New techniques are currently being developed which expand the capability of electrophoresis to detect genetic differences (Singh *et al.*, 1974; Johnson, 1976).

The simplest, most direct, and most informative measure of genetic variation from gene frequency data is the average proportion of heterozygotes per locus (i.e., average heterozygosity). This quantity can be calculated by directly counting the proportion of heterozygotes observed or by using the expected Hardy–Weinberg proportions and calculating the average proportion of heterozygotes using the observed gene frequencies.

In estimating the average heterozygosity (H) from populations there are a number of pitfalls that must be avoided to arrive at a reliable estimate of genetic variation. These considerations have been reviewed by Lewontin (1974). A critical factor is the number of loci examined; Nei and Roychoudhury (1974) have outlined the statistical procedures appropriate for estimating the variance of heterozygosity measures and in doing so have emphasized the importance of examining as many loci as possible.

Another serious problem is the type of loci used in heterozygosity estimates. It may be questioned whether the amount of genetic variation at the many other types of loci throughout the genome is reflected by that detected through isozyme loci. However, we believe that it is a reasonable assumption that the amount of variation at isozyme loci reflects the *relative* amounts of genetic variation found at other loci in the genome. We believe this to be true because many of the processes affecting the amount of genetic variation act uniformly on the genome (e.g., effective population size).

A more serious and avoidable error in estimating H is the predominant use of a single class of isozyme loci. Different classes of isozyme loci have been found to have consistently different amounts of variation over a wide range of organisms (Powell, 1975). The reliance on a single major class of isozyme loci (e.g., esterases) in the estimating of heterozygosity introduces a large bias into the estimate. Therefore, if

one's goal is to estimate heterozygosity in a population using isozyme data, one must strive to examine both a large number and wide range of isozymic loci.

In the course of our studies with salmonid populations we have accumulated a large amount of data which is appropriate for estimating average heterozygosity in several salmonid species. These estimates are presented in Table II. The average heterozygosity of these species ranges between 0 and 6%. The reliability of interspecific heterozygosity estimates has been challenged. The potential unreliability of such comparisons can be demonstrated by a simple example. Assume we estimate the average heterozygosity of a species based on some twenty genetic loci. If a twenty-first locus is added, which is polymorphic for two alleles with approximately equal gene frequencies, then our estimate of average heterozygosity will increase by approximately 2.5%. This example, taken into consideration with the

Table II

Average Heterozygosity in Nine Species of Salmonids[a]

Species	Common name	Number of populations	*H*	Range of *H*
Oncorhynchus				
O. gorbuscha	Pink salmon	6	0.039	0.032–0.047
O. keta	Chum salmon	5	0.045	0.043–0.048
O. kisutch	Coho salmon	10	0.015	0.000–0.025
O. nerka	Sockeye salmon	10	0.018	0.008–0.024
O. tshawytscha	Chinook salmon	10	0.035	0.024–0.052
Salmo				
S. apache	Apache trout	1	0.000	
S. clarki	Cutthroat trout			
	Coastal form	6	0.063	0.022–0.077
	Interior form	2	0.023	0.021–0.025
S. gairdneri	Rainbow trout	41	0.060	0.020–0.098
S. salar	Atlantic salmon	2	0.024	0.020–0.028

[a] These estimates are based on at least 30 loci in each species. These estimates were made by calculating the average heterozygosity in each individual population using allele frequencies, assuming Hardy–Weinberg proportions, and then averaging these estimates for all populations within a species. The following loci were used: *AAT-1*, -2, and -3; *AGPD-1* and -2; *ADH* (alcohol dehydrogenase); *GMP-1*, -2, and -3 (general muscle protein); *CPK-1* and -2 (creatine phosphokinase); *IDH-1* and -2 (isocitrate dehydrogenase); *LDH-1*, -2, -3, -4, and -5; *MDH-1*, -2, -3, and -4; *PGI-1*, -2, and -3; *PMI* (phosphomanose isomerase); *PGM-1* and -2; *6PGDH* (6-phosphoglucose dehydrogenase); *SDH-1* and -2 (sorbitol dehydrogenase); *SOD* (superoxide dismutase); *TFN*. From 50 to 100 individuals were examined in each population to estimate allele frequencies from that population.

narrow range of heterozygosities usually encountered in studies of natural populations (0–25%), should serve as warning on placing too much reliability on interspecific heterozygosity estimates.

We should point out, however, that the estimates of heterozygosity presented in Table II have been historically consistent and thus appear to be reliable. The sockeye salmon and rainbow trout were initially the most thoroughly examined species in our laboratory. Our initial observation, based on relatively few loci, was that the rainbow trout had a much higher amount of genetic variation than did the sockeye salmon. As additional loci have been added to our techniques over the years, this relationship has been extremely consistent.

Altukhov *et al.* (1972) have reported the average heterozygosity in chum salmon to be 3.2%; this estimate is very close to our estimate of 4.5%. They proposed that the low value of heterozygosity in chum salmon, relative to other species which have been studied, is the result of the great amount of gene duplication found in the salmonids. A look at H values in other salmonid species in Table II shows that a low amount of genetic variation is not the rule among salmonids. Therefore, the low amount of genetic variation found in some salmonids (e.g., sockeye salmon and coho salmon) cannot be readily explained by the presence of many duplicated genes, since some salmonid species do show a significantly larger amount of genetic variation.

Althukhov and his group have published a large amount of work with Asian salmonid populations which closely parallels our own work. Anyone interested in genetic variation in salmonids should be aware of the work that has been done on these species in the Soviet Union. We have included in the bibliography a selected listing of those papers by Altukhov's group of which we are aware (Altukhov *et al.*, 1975a,b).

The comparison of the amount of genetic variation between populations within a species is more reliable than interspecific comparisons. One reason for this reliability is that such estimates are not as sensitive to the inclusion of additional loci as are estimates for a species as a whole. This principle holds because a locus which is highly polymorphic in a particular population is usually polymorphic in most populations from that species. Therefore, the inclusion of an additional locus will tend to affect all populations similarly.

An interesting observation pertaining to Table II is that estimates of H for different populations within a species are remarkably consistent. To demonstrate this point, Table III shows the estimates of H from forty-one populations of rainbow trout. These populations are from four major sources: (1) natural populations of anadromous rainbow

Table III

Average Heterozygosities in Each of 41 Populations of Rainbow Trout[a]

Location[b]	Heterozygosity
Hatchery anadromous populations	
Big Creek, OR	0.058
Chelan, Columbia River, WA	0.050
Cowlitz River, WA (winter-run)	0.062
Cowlitz River, WA (summer-run)	0.068
Chambers Creek, WA	0.072
Deschutes River, OR	0.049
Clearwater River, ID	0.041
Pahsimeroi River, ID	0.048
Siletz River, OR	0.059
Snake River, ID	0.045
Wells Dam, Columbia River, WA	0.064
Washougal River, WA	0.070
Native anadromous populations	
Arnold Creek, WA	0.068
Deer Creek, WA (upstream)	0.056
Deer Creek, WA (downstream)	0.039
Clallam River, WA	0.071
Falls Creek, WA	0.071
Dickey River, WA	0.080
Gobar Creek, WA	0.086
Hoko River, WA	0.071
Kalama River, WA	0.056
Nooksack River, WA	0.050
Pysht River, WA	0.056
Quinault River, WA	0.062
Sauk River, WA	0.060
Soleduck River, WA (upstream)	0.056
Soleduck River, WA (downstream)	0.067
Stillaguamish River, WA (upstream)	0.053
Stillaguamish River, WA (downstream)	0.056
Twin River, WA	0.066
Wild Horse Creek, WA	0.067
Wishkah River, WA	0.080
Hatchery resident populations	
Chambers Creek, WA	0.060
Dream Lake, WA	0.056
Halle Sø, Denmark	0.098
Hoptrop, Denmark	0.067
Lem, Denmark	0.061
Mors, Denmark	0.075
Puyallup, WA	0.059
University of Washington, WA	0.020
Native resident population	
Chester Morse Lake, WA	0.055

[a] These estimates are based on those loci listed in Table II with the exception of *TFN*, which is polymorphic in rainbow trout but was not included because it was not examined in all populations (Allendorf, 1975).

[b] OR, Oregon; WA, Washington; ID, Idaho.

trout (i.e., steelhead), (2) hatchery populations of steelhead, (3) natural populations of nonanadromous rainbow, and (4) hatchery populations of rainbow trout which are maintained in the hatchery for their complete life cycle. The differences in the amount of genetic variation found in rainbow trout populations are discussed in the next section.

It is interesting to note the wide differences in H among species seen in Table II. There appears to be no simple explanation for these differences. As discussed before, the low H seen in some species cannot be simply explained by the extensive gene duplication in salmonids because of the high H seen in the rainbow trout, which has the same amount of gene duplication as other salmonid species. The low H seen in *Oncorhynchus* species is also not satisfactorily explained by the recent rapid evolutionary divergence of this group because of the similar low H in the Atlantic salmon, an old phylogenetic lineage which has not undergone recent divergence. Likewise, an explanation based on the relative effective population sizes of these species is not tenable. The sockeye salmon with low H is represented by immense populations in major river systems (e.g., the Fraser River and Bristol Bay drainages) while the steelhead (rainbow trout) is restricted to comparatively small population sizes throughout its range.

Whatever the cause of these differences in H among species, they are consistent and appear to be reflected in other ways. The rainbow trout has historically been the most successful salmonid species in adapting to new environmental conditions—whether in a hatchery or when planted in the wild. This greater adaptability of the rainbow trout is very likely due to the greater genetic variation throughout the genome as reflected by the high H values for this species measured with isozyme loci. The possible use of H as an indicator of the potential of a stock for genetic change is discussed in the next section.

C. Importance of Genetic Variation in Fish Culture

As emphasized earlier, genetic variation is required in a population if the attributes of that population are going to be changed via selection. Another factor which must be remembered is that this same genetic variability which makes change possible is decreased when such changes are accomplished. As stated by Gall (1972): "We must make every effort to learn and understand both the biology and genetics of the organism before we attempt to tamper with the essential but perishable resource, genetic variability" (p. 159).

The estimation of the additive genetic variability in a population

for a particular trait is difficult and involved. On the other hand, the estimation of the average amount of genetic variation in a population of isozyme loci is simple and straightforward. We would like to point out a possible relationship between these two measures of genetic variation.

We submit that a high amount of genetic variability as measured by isozyme loci is an indicator of high genetic variability throughout the genomes of that population. Therefore, populations with high average heterozygosities should also demonstrate high additive genetic variance for phenotypic traits which are of importance to fish culturists. Although this is currently only an hypothesis, it is one that can and should be tested; both parameters involved can be directly estimated.

The practical importance of such a relationship is great. One would be able to predict the relative success of a particular stock undergoing a program of artificial selection. In addition, one could create a stock with exceptionally high genetic variation by forming a new stock composed of individuals from populations which are initially extremely genetically different. Therefore, using average heterozygosity as an indicator of potentially valuable genetic variation, one could evaluate the potential of a current stock for genetic change, select a stock to use in a selection program which has an initial great deal of genetic variation, or create new stocks with exceptionally high genetic variation.

A word of caution must be interjected at this point. Estimating genetic variation by isozyme frequencies cannot replace the value of estimating the heritability of particular traits. When developing a program of selection one must consider the comparative heritability of particular traits to be selected. However, estimates of variability based on isozyme data allows one to predict the expected success to be obtained using different populations within a single species.

The contention has often been made that the artificial propagation of salmonid populations has led to the reduction of genetic variability in such populations. This contention can now be tested by examining the amount of genetic variation in different populations within a species as measured with isozymes. An examination of natural and hatchery populations of anadromous rainbow trout in Table III shows that there is no indication of loss of genetic variation in these hatchery populations. Thus, the procedures in developing and maintaining these populations in a hatchery during part of their life cycle has not resulted in a loss of genetic variation.

The examination of hatchery populations of rainbow trout which are completely maintained in a hatchery reveals a different situation, however. Eight populations of nonanadromous rainbow trout are

shown in this table. The estimates of H in seven of these populations are between 5.6 and 9.8%. The exceptional population is the University of Washington hatchery stock which has an H value of 2.0%. Seven of the hatchery populations show no evidence of a reduction in genetic variation. However, the dramatic reduction in H of the University of Washington stock seems to indicate a significant loss in genetic variation in this stock. This stock has been maintained in the hatchery for a period of 40 years, during which it has been subjected to both a reduced population size and an ongoing program of mass selection for several characters, for example, body size, egg production, and age at maturity (Donaldson and Olson, 1955). Thus, it appears that this program has caused a drastic reduction in the amount of genetic variability in this stock. Such a reduction is potentially deleterious to the production characteristics of a stock. Studies with a variety of cultured organisms have shown that reduction in genetic variability such as this very often results in reduced viability of the population. The recently reported (Hershberger *et al.*, 1976) poor survival of individuals from this stock during the early life of the zygote may be attributable to this loss of genetic variation.

The loss of genetic variation because of hatchery procedures is of concern to all those involved in the maintenance of hatchery populations of fish. An awareness of the factors involved in the loss of genetic variation in such a manner is extremely important. The reader is encouraged to pursue such sources as Falconer (1960) and Crow and Kimura (1970) to acquire such an understanding. We will present a brief review, however, of the important factors involved.

The loss of genetic variation in artifically cultured populations of plants and animals because of inbreeding is well known. However, the actual genetic processes underlying this phenomenon are often misunderstood. "Inbreeding" is a term which is used to describe a variety of different circumstances (Jacquard, 1975).

In the present context, the loss in genetic variation is largely attributable to a limited population size. The magnitude of this loss in a single generation is inversely proportional to the size of that population as expressed by the following equation

$$\Delta F = \frac{1}{2N_e}$$

where F is the inbreeding coefficient, which represents the proportional loss in genetic variation, and N_e is the effective size of the population (Crow and Kimura, 1970).

The effective size of a population is rarely equal to the total

number of reproductive individuals in that population. Rather, the effective population size is defined as the size of an ideally behaving population that would have the same decrease in genetic variation as the observed population. A major factor which influences N_e is the relative number of males and females in a population. The effective population size of a population with different numbers of males and females is expressed by the relationship

$$\frac{1}{N_e} = \frac{1}{4N_f} + \frac{1}{4N_m}$$

where N_f and N_m are the numbers of females and males in the population, respectively (Crow and Kimura, 1970). The important consideration is that the value of N_e is strongly influenced by the sex which is the least frequent. For example, a population consisting of 1 male and 1 female has an N_e of 2, while a population with 1 male and 100 females has an N_e of approximately only 4.

Another major factor in the loss of genetic variation is fluctuations in population size from generation to generation. A greatly reduced population size for a single generation can have a drastic effect. Such bottlenecks should be avoided in the propagation of hatchery stocks.

Loss of genetic variation because of limited population size can be avoided given the proper concern and an understanding of the principles involved. Our results have shown that such a loss has occurred in at least one hatchery stock of rainbow trout. The potential estimation of genetic variation in a stock via isozyme examination provides a means to assess any possible loss of genetic variation in a hatchery stock. This capability may prove extremely valuable in estimating the potential value of a particular stock.

V. THE USE OF GENETIC DATA IN FISHERY MANAGEMENT

This section is based on some of our applications of electrophoretic data in the management of fish populations. These examples are drawn from our own experience but the concepts are much more generally applicable. The reader is also referred to the following sources for other discussions of applying genetic principles in the management of fish populations (Calaprice, 1969, 1970, 1976; Rasmuson, 1968; Purdom, 1972, 1976).

The capability of managing a fishery on the basis of its component populations is an objective that has generally eluded salmonid biologists until recently because of the difficulties involved in defin-

ing these populations. Tagging and marking studies have provided useful information concerning origins and degrees of straying of fish but have been limited by the need for handling all treated individuals. Natural features such as scale characters and relative mineral composition have also proven actually or potentially useful for identification of areas of origin through reflections of natal environments. None of the above approaches are capable of genetically defining population structures, however, and such definitions are necessary if management is to be based on population structures.

Properly selected electrophoretic data can genetically define populations on the basis of gene frequencies at different loci. Such data permit estimation of relative genetic similarity among populations of a species in the same manner that similarity among species can be estimated. Frequencies of variants in a given population are stable attributes of that population and tend to persist at the same level over many generations in salmonid populations that we have studied; estimates are therefore usually cumulative over time and may be added together to provide greater precision rather than requiring redefinition every year.

As a result of these attributes, electrophoretic studies of salmonid populations of the Pacific Northwest carried out by our unit and other groups have genetically defined these populations in much greater detail and clarity than had previously been possible. The overall picture is far from complete because of the complexities of salmonid populations, but an overview of major population units can be presented for some species on the basis of data that has been collected to date. This section presents such an overview, and discusses some areas of fish culture where we believe that electrophoretic data are particularly applicable.

A. Species Identification

Adult salmonid species are usually unambiguously identifiable on the basis of external morphology, particularly during the time of spawning. However, morphological identification of very young salmonids is often not possible. Such individuals are readily identifiable to species from biochemical genetic data obtained from freshly collected material (Fig. 3). This capability permits precise species assessment in stream surveys, even at very early life history stages; it has proven particularly useful for identification of cutthroat and rainbow trout, which often occur sympatrically and are morphologically very

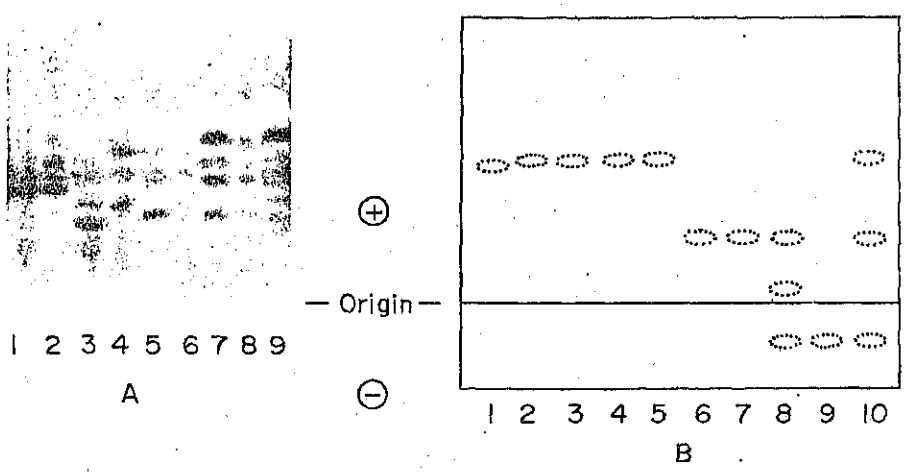

Fig. 3. Isozyme patterns useful in identification of nine salmonid species of two genera. Each species can be unambiguously identified through combined use of these two systems alone. (Note expression of bands from both parent species in hybrids.) (A) Creatine kinase (from muscle): 1, cutthroat trout; 2, rainbow trout; 3, masu salmon (*O. masou*); 4, sockeye salmon; 5, chum salmon; 6, pink salmon; 7, coho salmon; 8, chinook salmon; 9, chinook–masu salmon hybrid. (B) Superoxide dismutase (from liver): 1, chum salmon; 2, sockeye salmon; 3, masu salmon; 4, rainbow trout; 5, cutthroat trout; 6, coho salmon; 7, pink salmon; 8, pink–chinook salmon hybrid; 9, chinook salmon; 10, masu–chinook salmon hybrid.

similar as juveniles. Hybrids among the salmonid species that we have studied are usually clearly identifiable through biochemical genetic markers; we have often assisted other workers by identifying the appropriate ancestry of suspected hybrid individuals.

The absence of qualitative biochemical genetic differences among nominal species is insufficient evidence to demonstrate that these populations are conspecific, because sufficient genetic differences for reproductive isolation may exist at unsampled loci. Such data, of course, do not preclude conspecificity of the populations in question and are supportive of positive evidence derived by other means. A study of twenty biochemical genetic loci in rainbow trout, California golden trout (*S. aguabonita*), and red-banded trout (*Salmo* sp.)—a group of trout indigenous to dessicated basins of eastern Oregon (Behnke, 1965; Wilmot, 1974)—indicated that these three natively allopatric groups shared common alleles at every locus (Allendorf and Utter, 1974). Conspecificity of these three groups of trout is also supported by the tendency of rainbow and golden trout to hybridize readily when placed in the same drainages (Behnke, 1965). The question of species remains unresolved, however, because of the tendency of

rainbow trout and cutthroat trout, two valid species, also to hybridize under some conditions (Behnke, 1965). Management of these three groups of trout should emphasize continued separation if their identities are to remain distinct (Behnke, 1965).

B. Identification of the Genetic Structure of Natural Populations

A major goal of our studies is the genetic characterization of salmonid populations of the Pacific Northwest. Fulfillment of this goal can provide major insights into the breeding structure of these species and lead to a sound basis for identification of areas of origin in mixed fisheries. The problem has been complicated by the transplantation of stocks from one area to another, but sufficient variation persists among major groups within species to characterize these groups on the basis of frequency differences of biochemical genetic markers. We summarize here some of our more extensive studies of either natural populations or hatchery populations derived principally from native fish from the area of the hatchery. The data were obtained over a number of years and appear to reflect stable genetic attributes of the populations studied (regardless of whether the variation is maintained by random or selected processes).

An electrophoretic survey of 32 loci of anadromous rainbow trout (i.e., steelhead) populations of the Pacific Northwest (Allendorf, 1975) revealed considerable genetic heterogeneity among them and indicated some relationships that had not previously been known (Fig. 4). A major division occurs among populations at a point coinciding with the crest of the Cascade Mountains (Fig. 5).

A similar east–west division of LDH variants has also been reported for both migratory and nonmigratory rainbow trout on the Fraser River (Huzyk and Tsuyuki, 1974). Two major taxonomic units were proposed for rainbow trout of this area on the basis of these findings, a coastal group and an inland group. The inland group presumably descended from rainbow trout residing in large lakes formed from inland drainages of the Fraser and Columbia Rivers during the last period of glaciation (McKee, 1972). The coastal areas were apparently repopulated by another group—possibly the Asiatic rainbow trout (*S. mykiss*) (Behnke, personal communication)—when the glaciers receded. The evidence indicating that geographic separation is the principle basis for genetic isolation of rainbow trout populations differs from previous conceptions in which anadromy and time of return to freshwater were regarded as the primary indications of genetic differences (Behnke, 1965; Withler, 1966; Millenbach, 1973).

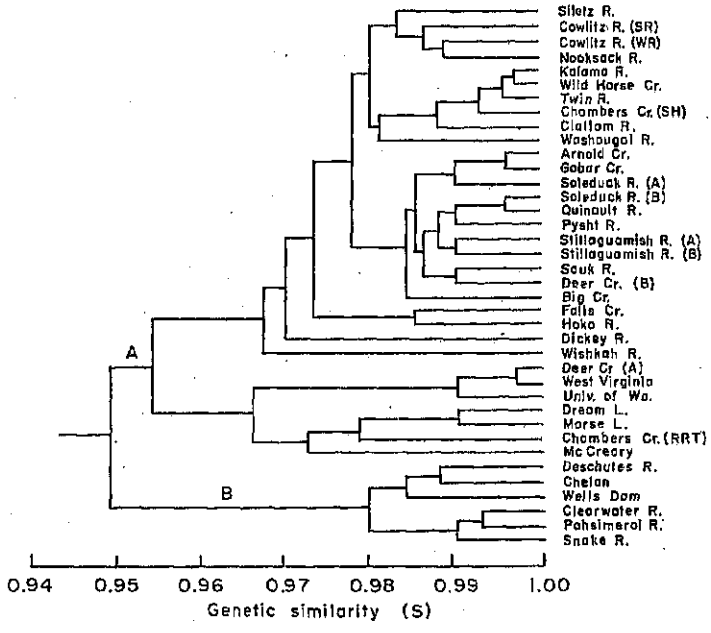

Fig. 4. Dendrogram (unweighted average linkage method) of genetic similarity among 38 populations of rainbow trout based on Rogers (1972) similarity coefficients obtained from gene frequencies at 32 loci. The five populations contained in branch B represent the inland group of rainbow trout found east of the Cascade Crest. All those populations in branch A are representatives of the coastal form. (From Allendorf, 1975.)

Coho salmon of the Pacific Northwest have been examined in sufficient detail to identify some major population units. Populations of both the Columbia and Fraser Rivers and their tributaries are distinguished by high frequencies of the gene coding for the most anodal form (*TFN-103*) of the serum protein transferrin (Utter *et al.*, 1970, 1973; May, 1975). Coho salmon populations returning to other areas between and on either side of these two large river systems are characterized by a predominance of the most cathodal migrating form of transferrin (*TFN-97*) and the presence of a third form of intermediate electrophoretic mobility (Fig. 5). The discontinuous distribution of transferrin alleles among coho salmon populations cannot be directly explained on the basis of glacial events (as could discontinuities of rainbow trout populations of these rivers) because the predominance of the *TFN-103* allele extends the full length of both large rivers. This distribution may be related to environmental factors of the large rivers, a possibility which is currently being investigated.

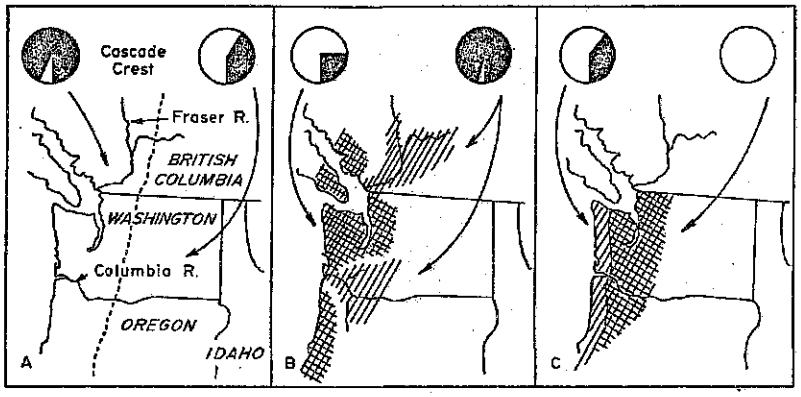

Fig. 5. Major population groups in three anadromous salmonid species of the Pacific Northwest. (A) Average proportion of *LDH-4 (100)* allele in rainbow trout (represented by shaded areas of circles) in areas east and west of the Cascade Crest in Washington, Oregon, Idaho, and southern British Columbia. (B) Average proportion of *TFN (103)* allele of coho salmon (represented by shaded areas of circles) in coastal and Puget Sound drainages of Washington, Oregon, and British Columbia (checked areas), and Columbia River and Fraser River drainages (lined areas). (C) Average frequencies of *PGI-2(68)* allele of fall chinook salmon (represented by shaded areas of circles) in Puget Sound and Columbia River drainages (checked areas) and coastal populations (lined areas) of Washington and Oregon.

Populations of chinook salmon returning to fresh water in the fall have been examined from Puget Sound, along the Washington and Oregon coasts, and in the Columbia River (Utter *et al.*, 1973; Kristiansson, 1975; May, 1975). Coastal populations of Washington and Oregon have high frequencies of variants of PGI and also usually have phosphoglucomutase (PGM) variation; both PGI and PGM are invariant in samples taken from Puget Sound and the Columbia River (Fig. 5). These differences indicate considerable genetic isolation of coastal and noncoastal populations of fall-run chinook salmon and are potentially very useful for determining major areas of origin of ocean caught fish.

These biochemical genetic studies of salmon and trout populations have defined major natural population units that had not been previously identified. This knowledge is presently being used in the management of these fisheries. Allocation to one or another major grouping in mixed fisheries can be made using known gene frequencies from the major groups and gene frequencies obtained from sampling of the fisheries. An actual example of this application is outlined in Table IV for coho salmon collected in the Pacific Ocean near Neah Bay, Wash-

Table IV

Estimated Proportions of Columbia and Fraser Rivers Fish in Samples of Coho Salmon Caught in the Pacific Ocean near Neah Bay, Washington[a]

Collection number	Collection date	Sample size	Frequency of *TFN-103* in ocean catch (F_m)	Estimated proportion of Columbia and Fraser Rivers fish in sample (p)
1	25 Jul 75	53	0.537	0.39
2	12 Aug 75	106	0.425	0.25
3	8 Sep 75	110	0.464	0.30
4	11 Sep 75	114	0.504	0.35

[a] These estimates were calculated assuming fixation of the *TFN-103* allele in the Columbia and Fraser Rivers and an average frequency of the *TFN-103* allele of 0.250 estimated from 59 collections from other areas of Washington State. The proportion (p) was calculated by $p = (F_m - F_w)/(F_{fc} - F_w)$, where F_m, F_{fc}, and F_w are the frequencies of *TFN-103* in the ocean catch, Columbia and Fraser Rivers, and Washington State coastal and Puget Sound streams, respectively.

ington, during the fall of 1975. The data indicate that a sizable proportion of this fishery is supported by the two large river systems.

These data can also be used to suggest modifications of current management practices. Steelhead returning to the Columbia River in the summer have previously been regarded as a single genetic unit, contrasted with those returning in the winter (Millenbach, 1973). Planting of summer-run steelhead derived from coastal fish in inland areas (where all steelhead are summer-run fish) have been unsuccessful (personal communication, Washington State Department of Game). This failure is understandable now that the major grouping of steelhead appears to be based on inland and coastal populations rather than time of return, and it is not surprising that coastal fish were not successful when planted in inland areas.

Statistical differences also exist among natural populations within major subgroupings of these salmonid species. For instance, coho salmon populations in south Puget Sound are characterized by average gene frequencies of a variant LDH allele of about 0.05; this allele is virtually absent in north Puget Sound populations (May, 1975). Differences among coastal steelhead populations are seen in the frequencies of alleles at many loci. These variations are generally more subtle than those observed between major subgroups; they are useful in defining populations and are potentially applicable in identifying component populations of mixed fisheries. The application in the latter case is complicated by multiple genetic variants and populations beyond the

simple instance outlined in Table IV. Analysis is feasible, provided appropriate baseline data are available, by fitting the appropriate data parameters through computerized methods using the techniques of maximum likelihood and minimum χ^2 (Kempthorne, 1957; Krieger *et al.*, 1965). Personnel of our group are presently developing and applying this analytical capability.

It is interesting at this point to consider the quite different population structures of these three closely related sympatric species which have similar life histories. Each species has its own major population groupings which are defined through distinct arrays of polymorphic loci. Although some possible explanations for a particular distributional pattern within a species have been suggested above, possible reasons for such diversity among species remain obscure. It seems likely that many factors have interacted to bring about these differences including sequence of initial entry to the region, differential tendencies of straying from natal streams, and differential adaptive capabilities of a species to a particular region. Regardless of cause, it is apparent that generalizations regarding population structure cannot be safely made even among closely related species; and it follows directly that management practices that are based on population structures must arise from direct evidence rather than inferential data from closely related species.

C. Hatchery Populations

The emphasis to this point has concerned the management applications of protein variants of natural populations. The focus now shifts to hatchery populations.

Determining the effects of plantings of hatchery fish on native salmonids of the same species is a major concern to management biologists. Native fish are a valuable reservoir of genetic variation and provide a useful supplement to the fishery, even in stocks that are largely maintained through hatcheries. Although native fish may be more adapted to a particular area than hatchery fish, they are potentially endangered through hatchery plantings by factors including (1) competition for spawning and rearing grounds resulting from large hatchery releases, (2) possible earlier hatching of progeny of hatchery fish resulting in a competitive advantage, and (3) hybridization of native and hatchery fish resulting in disruption of adaptive gene pools.

Biochemical genetic markers are very useful for studying the effects of hatchery plantings on native fish, provided there are differ-

ences in gene frequencies between the two groups. Genetically marked hatchery fish require no special handling prior to release, and long-term effects of plantings can be measured because genetic markers are passed on to subsequent generations.

We are presently collaborating with the Washington State Department of Game in studying the effects of plantings of two stocks of hatchery steelhead maintained by the department which have been introduced in certain rivers of Washington State. The Kalama River, a tributary of the lower Columbia River, has been heavily planted in recent years with summer-run steelhead from the department's hatchery on the Washougal River, which enters the Columbia River 30 miles upstream from the Kalama. Native fish from the Kalama River and winter-run hatchery fish that have been planted in the river both lack genetic variants for AGPD while the Washougal hatchery stock has a variant form of the enzyme with a gene frequency of about 0.15. This difference has been useful in tracing the effects of plantings of Washougal hatchery fish in the Kalama River and other river systems of western Washington.

Data from these preliminary studies indicate some interesting interactions of Washougal hatchery fish with other steelhead stocks of the Kalama River. Hatchery fish planted in the main stream of the Kalama River tend to enter tributaries prior to their seaward migration. Adult fish from hatchery plantings return near the point of release and many of them spawn successfully. Descendants of these fish apparently hatch earlier than those of other stocks based on their larger size in a given sampling area (although existing data cannot exclude other factors such as faster growth rates). Almost all of the residualized steelhead (i.e., fish that remain in the river rather than migrating to sea) appear to be from the Washougal hatchery. These data indicate considerable long-term competition of Washougal hatchery fish with the native steelhead stocks of the Kalama River. Plans for more detailed studies of this competition are outlined below.

D. Genetic Marking of Stocks

The potential value of a genetic marker for the identification of populations increases as the differences in its frequency increases between populations. The sample size needed to demonstrate differences between two populations decreases to the point where individual fish can be identified if different alleles for a particular protein are fixed in the two populations. Such a situation rarely occurs naturally

within a species, particularly among populations where gene flow is possible, but can be created through artificial propagation.

We are presently working with the Washington State Department of Game to create genetically marked stocks for maximizing genetic differences between these stocks and native fish in areas where the stocks are to be planted. One such stock is being bred from Washougal hatchery fish for introduction into previously unplanted tributaries of the Kalama River. Breeding is based only on the AGPD variation. Fish are selected for breeding by a screening process involving muscle biopsy, tagging, and electrophoresis of muscle samples. The breeding scheme is outlined in Table V. Initial selection based on homozygous males [*AGPD (140/140)*] bred with randomly selected females will be repeated each year for 4 years (we have presently completed 2 years). After that time the progeny of the first-year crosses will return as adults to the point of release; this point is a previously unstocked pond where only selected progeny have been reared. These fish will be screened for *AGPD (140/140)* individuals of both sexes to be used exclusively as breeders, and this procedure will also be repeated each year for 4 years. The *140* allele will now be fixed in this stock and spawning fish returning to the site of release can be spawned randomly. Fish returning to the pond should be screened periodically to assure that a significant influx of unmarked fish does not enter the spawning population.

Table V

Breeding Scheme for Fixation of *AGDP-140* Allele in a Derivative Stock of Washougal River Hatchery Anadromous Rainbow Trout

	AGPD				
	Genotype frequencies			Allele frequencies	
	100/100	*100/140*	*140/140*	*100*	*140*
Parental population					
Total population	0.72	0.25	0.02	0.85	0.15
Breeding males	0	0	1.00	0	1.00
Breeding females	0.72	0.25	0.02	0.85	0.15
First generation after selection					
Total population	0	0.85	0.15	0.42	0.58
Breeding males	0	0	1.00	0	1.00
Breeding females	0	0	1.00	0	1.00
Final derivative stock					
Total population	0	0	1.00	0	1.00

There are two potential genetic pitfalls that must be kept in mind in a breeding scheme of this kind. The first of these is the possibility that the variant form of AGPD (or any other protein that might be selected) has a selective disadvantage contrasted with the common form of the enzyme. If such a disadvantage exists, the selected stock would be less genetically fit than the parent stock and conclusions drawn from the selected stock pertaining to the parent stock would be biased. It is therefore important to select a marker that is not obviously associated with any negative characteristic, and to carry out controlled tests on selected and parent stocks to be reasonably sure that both stocks are comparable for measurable variables other than the selected marker. This danger is especially present when selecting for an allele which is very rare in the original population. An allele present at an original frequency of less than 0.01 is much more likely to have a potential harmful effect than an allele which is present at a frequency of 0.15 and is therefore already present in 25% of the fish in the original population (assuming Hardy–Weinberg proportions). We have not detected any differences among the three AGPD phenotypes of the Washougal stock, and relative attributes of the parent and selected stocks are being monitored.

The other genetic potential pitfall that must be remembered is inbreeding (see Section IV,C). Inbreeding depression is a loss of vigor that occurs in most sexually reproducing organisms as a result of the reduction of genetic variation accompanying breeding of closely related individuals. This depression reflects factors including the expression of deleterious recessive genes, and the reduction of beneficial interactions both within and between loci. Inbreeding coefficients above 0.10 (this represents a loss of 10% of the genetic variation present in the original population) are often sufficient to result in detectable loss of vitality. The inbreeding coefficients induced in founder populations originating from 100 females and 1–10 males are plotted in Fig. 6. It is apparent that use of a single male would create a potential danger to the stock from inbreeding, and that this danger is reduced dramatically as up to 6 additional males are used—where a leveling off point is reached. Initial inbreeding is not significantly increased in subsequent generations provided selection of the parents is randomized and adequate numbers of individuals, say 50 or more of each sex, are bred. Both of these potential sources of genetic weakness must be anticipated in planning a genetic marking program but neither represents a serious obstacle if caution is exercised.

We foresee artificial genetic marking of hatchery stocks becoming a very useful management tool. The breeding scheme outlined in Table

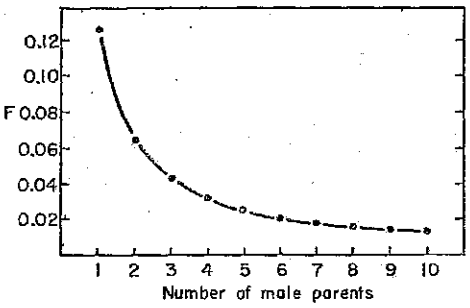

Fig. 6. Coefficients of inbreeding (F) after one generation of mating based on 100 female parents and varying numbers of male parents. $F = (N_m + N_f)/8N_mN_f$, where N_m, number of male parents, and N_f, number of female parents, equal 100.

V was a special case of an anadromous stock being brought to fixation with males only being selected in the first generation. The process can be simplified in hatcheries where brood stock are maintained because the same individuals can be used on successive years. If fixation of a marker gene is not required, useful gene frequency changes can be achieved in a single generation even if only males are selected. Change of gene frequency can obviously be accelerated if initial selection for both sexes is feasible.

Genetically marked hatchery stocks have many management applications in addition to evaluating the effect of hatchery plantings on native fish populations. Any management program based on differential harvest of a particular anadromous fishery would be much easier to implement if specific segments of the fishery were genetically marked. It has been indicated above that sufficient genetic differences already exist among major population units to permit some allocation of component stocks of mixed fisheries. Hatchery marking would significantly enhance this capability by creating additional differences where none presently exist.

Genetic marking of hatchery fish also has a significant potential for application in nonanadromous fisheries. Sufficient biochemical genetic variation appears to be present in most trout species to permit differential marking of all hatchery strains that may be planted in a particular region. The results of particular plantings can be closely followed in closed stream or lake systems and the performance of different hatchery strains can be precisely evaluated in the same environment. Measurements of such factors as relative survival, growth, reproduction, and dispersal can be readily obtained.

The above discussion has mentioned a number of applications of genetically marked hatchery stocks. Many additional uses of the method certainly exist and, of course, the concept can be generally applied to any cultured species. We anticipate that genetic marking will ultimately become a routine aquacultural procedure and urge management and hatchery personnel to seriously consider the process in their future planning.

VI. SUMMARY

We have reviewed genetic studies of fish populations emphasizing electrophoretic studies because the overwhelming majority of knowledge pertaining to genetics of fish populations has come from this source. Fish are especially appropriate as a group for studies of genetics of natural populations because of their extreme diversity, large population sizes, and their poikilothermic physiology which increases their susceptibility to thermal effects of the environment. Fish are also widely cultured, and there are many aspects of population genetics that are directly applicable to cultured populations of fish.

A discussion of isozyme methodology focuses on the genetic interpretation of electrophoretic variation. Use of multiple buffer systems, multiple tissues, and a wide variety of staining procedures are necessary to maximize the amount of genetic data that can be detected in a given species. Much electrophoretic variation is nongenetic and it is essential to identify only genetic variation in order to preclude a faulty data base for an investigation. The best criterion for a genetic basis of a variant is actual breeding data although alternate criteria may be reliably invoked if breeding studies are not feasible. Multiple systems of nomenclature of isozymic variants of fish have led to ambiguities and confusion. A unified system of nomenclature is proposed which would minimize this confusion and which is based on a numerical designation of allelic variants where the most common form is arbitrarily used as a standard.

A section is devoted to major areas of isozyme investigations of fishes. Much effort has been directed towards the measurement of natural selection. Extensive studies of eelpouts have indicated that demonstration of selection is itself very difficult, and even when demonstrated, may still not be informative regarding the method of maintenance of polymorphism. Relationships have been demonstrated in some fish species between geographic patterns of protein variation

and the functional properties of a protein. Such correlations are evidence for selection but do not necessarily constitute proof; electrophoretically identical but functionally different proteins must also be considered, as well as the possibility of groups of co-adapted loci. Studies of eels and pink salmon are examples of well-planned investigations that respectively provide evidence for and against selection as the primary method of maintenance of polymorphisms in a species; more studies of this kind are encouraged. Systematic studies based on isozymic data have provided a useful complement to studies of the same taxa where other criteria have been used. Exceptional instances tend to reinforce the value of using protein data in systematic studies.

A discussion of genetic variation in populations of fish puts forth the concept that the relative amount of genetic variation in particular populations within a species is an indicator of the potential of that stock to undergo genetic change in a program of artificial selection. A review of the amount of genetic variation based on our own studies shows that (1) different species of salmonids have significantly different amounts of genetic variation as measured by average heterozygosity, (2) populations within a species have remarkably similar levels of heterozygosity, and (3) hatchery management procedures, in at least one case, have resulted in the loss of genetic variation in artificially cultured stocks. We also emphasize the role of limited population size in causing the loss of genetic variation in hatchery populations of fish.

Protein variations have been applied to define genetically some populations of fishes in much greater detail than had previously been possible. This capability offers some new possibilities for fisheries management. Biochemical identification of species can frequently be made on samples where morphological criteria are not useful. Identification of population groups provides data for management based on the genetic structure of these populations. Proportions of component populations of mixed fisheries can be determined if known differences in frequencies of isozyme variants exist among these populations. Biochemical genetic differences among hatchery populations can be maximized through selection for specific protein variants. Such genetic marketing can be done without affecting performance characteristics of a particular stock. This procedure has considerable potential as a tool in the management of fish populations.

Note. This chapter reviews research on population genetics of fish conducted prior to August 1976 (the date the chapter was written).

ACKNOWLEDGMENTS

F.W.A. worked on the initial stages of this chapter while at the Department of Genetics, Aarhus University, Aarhus, Denmark, and he gratefully acknowledges the support given to him by that department and by the Danish Science Council.

We would like to thank the following agencies for their cooperation and assistance during the course of our studies: the U.S. National Marine Fisheries Service; the Washington Cooperative Fisheries Unit, College of Fisheries, University of Washington; the Washington State Departments of Game and Fisheries; the Alaskan Department of Fish and Game; the U.S. Fish and Wildlife Service; and the Oregon Cooperative Fisheries Unit, Oregon State University.

REFERENCES

Allendorf, F. W. (1975). Genetic variability in a species possessing extensive gene duplication: Genetic interpretation of duplicate loci and examination of genetic variation in populations of rainbow trout. Ph.D. Thesis, Univ. of Washington, Seattle.

Allendorf, F. W., and Utter, F. M. (1973). Gene duplication within the family Salmonidae: Disomic inheritance of two loci reported to be tetrasomic in rainbow trout. *Genetics* 74, 647–654.

Allendorf, F. W., and Utter, F. M. (1974). Biochemical genetic systematics of the genus Salmo. *Anim. Blood Groups Biochem. Genet.* 5, Suppl. 1, p. 33. (Abstr.)

Allendorf, F. W., and Utter, F. M. (1976). Gene duplication in the family Salmonidae. III. Linkage between two duplicated loci coding for aspartate aminotransferase in the cutthroat trout (*Salmo clarki*). *Hereditas* 82, 19–24.

Allendorf, F. W., Utter, F. M., and May, B. P. (1975). Gene duplication within the family Salmonidae. II. Detection and determination of the genetic control of duplicate loci through inheritance studies and the examination of populations. *In* "Isozymes," Vol. 4, "Genetics and Evolution" (C. L. Markert, ed.), pp. 415–432. Academic Press, New York.

Allendorf, F. W., Ryman, N., Stennek, A., and Stahl, G. (1976). Genetic variation in Scandinavian populations of brown trout (*Salmo trutta* L.): Evidence of genetically distinct sympatric populations. *Hereditas* 83, 73–82.

Altukhov, Y. P. (1974). "Populyatsionnaya Genetika Ryb" (Population Genetics of Fishes). Pishchevaya Promyshlennost' Press, Moscow. [Transl., Fish. Mar. Serv. Transl. Ser. No. 3548 (1975).]

Altukhov, Y. P., Salmenkova, E. A., Omelchenko, V. T., Sachko, G. D., and Slynko, V. I. (1972). The number of mono- and polymorphous loci in the population of the tetraploid salmon species *Oncorhynchus keta* Walb. *Genetika* 8(2), 67–75.

Altukhov, Y. P., Salmenkova, E. A., Konovalov, S. M., and Padoukin, A. I. (1975a). Stationary distributions of frequencies of lactate dehydrogenase and phosphoglucomutase genes in population system of local fish stock *Onchorhunchus nerka* I. Stability of the stock in generations under simultaneous variability of subpopulations making up their structure. *Genetika* 11(4), 44–53.

Altukhov, Y. P., Pudovkin, A. I., Salmenkova, E. A., and Konovalov, S. M. (1975b). Stationary distribution of frequencies of lactate dehydrogenase and phosphoglucomutase genes in population system of local fish stock, *Onchorhynchus nerka*.

II. Random genetic drift, migration, and selection as factors of stability. *Genetika* 11(4), 54–62.

Amend, D., and Smith, L. (1974). Pathophysiology of infectious hematopoietic necrosis virus disease in rainbow trout (*Salmo gairdneri*): Early changes in blood and aspects of the immune response after injection of IHN virus. *J. Fish. Res. Board Can.* 31, 1371–1378.

Aspinwall, N. (1974). Genetic analysis of North American populations of the pink salmon, *Oncorhynchus gorbuscha*, possible evidence for the neutral mutation–random drift hypothesis. *Evolution* 28, 295–305.

Aulstad, D., Gjedrem, T., and Skjervold, H. (1972). Genetic and environmental sources of variation in length and weight of rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Board Can.* 29, 237–241.

Avise, J. C. (1974). Systematic value of electrophoretic data. *Syst. Zool.* 23, 465–481.

Avise, J. C., and Kitto, G. B. (1973). Phosphoglucose isomerase gene duplication in the bony fishes: An evolutionary history. *Biochem. Genet.* 8, 113–132.

Avise, J. C., and Smith, M. H. (1974). Biochemical genetics of sunfish. II. Genetic similarity between hybridizing species. *Am. Nat.* 108, 458–472.

Avise, J. C., Smith, J. J., and Ayala, F. J. (1975). Adaptive differentiation with little genic change between two native California minnows. *Evolution* 29, 427–437.

Ayala, F. J. (1975). Genetic differentiation during the speciation process. *In* "Evolutionary Biology" (T. Dobzhansky, M. K. Hecht, and W. C. Steere, eds.), Vol. 8, pp. 1–78. Plenum, New York.

Behnke, R. J. (1965). A systematic study of the family Salmonidae with special reference to the genus Salmo. Ph.D. Thesis. Univ. of California, Berkeley.

Behnke, R. J. (1973). The rationale of preserving genetic diversity: Examples of the utilization of intraspecific races of salmonid fishes in fisheries management. Colorado State Univ., Ft. Collins.

Boyer, S. H. (1972). Extraordinary incidence of electrophoretically silent genetic polymorphisms. *Nature (London)* 239, 453–454.

Brewbaker, J. L. (1964). "Agricultural Genetics." Prentice-Hall, Englewood Cliffs, New Jersey.

Calaprice, J. R. (1969). Production and genetic factors in managed salmonid populations. *In* "Symposium on Salmon and Trout in Streams" (T. G. Northcote, ed.), pp. 377–388. Inst. Fish., Univ. of British Columbia, Vancouver.

Calaprice, J. R. (1970). Genetics and mariculture. *Fish. Res. Board Can. Tech. Rep.* No. 222.

Calaprice, J. R. (1976). Mariculture—ecological and genetic aspects of production. *J. Fish. Res. Board Can.* 33, 1068–1084.

Cavalli-Sforza, L. L. (1974). The genetics of human populations. *Sci. Am.* 231, 81–89.

Chen, S. H., Fossum, B. L. G., and Giblett, E. R. (1972). Genetic variation of the soluble form of NADP-dependent isocitric dehydrogenase in man. *Am. J. Hum. Genet.* 24, 325–329.

Cherfas, B. I., ed. (1972). "Genetics, Selection, and Hybridization of Fish." (Transl. from Russ., Isr. Program Sci. Transl., Jerusalem; available from U.S. Dep. Comm., Nat. Tech. Inf. Serv., Springfield, Virginia.

Christiansen, F. B., and Frydenberg, O. (1973). Selection component analysis of natural polymorphisms using population samples including mother–offspring combinations. *Theoret. Pop. Biol.* 4, 425–445.

Christiansen, F. B., Frydenberg, O., and Simonsen, V. (1973). Genetics of *Zoarces* popu-

lations. IV. Selection component analysis of an esterase polymorphism using population samples including mother–offspring combinations. *Hereditas* 73, 291–304.
Christiansen, F. B., Frydenberg, O., Gyldenholm, A. O., and Simonsen, V. (1974). Genetics of *Zoarces* populations. VI. Further evidence, based on age group samples of a heterozygote deficit in the *EST III* polymorphism. *Hereditas* 77, 225–236.
Clayton, J. W., and Tretiak, D. N. (1972). Amine citrate buffers for pH control in starch gel electrophoresis. *J. Fish. Res. Board Can.* 29, 1169–1172.
Clayton, J. W., Tretiak, D. N., Billeck, B. N., and Ihssen, P. (1975). Genetics of multiple supernatant and mitochondrial malate dehydrogenase isozymes in rainbow trout (*Salmo gairdneri*). *In* "Isozymes," Vol. 4, "Genetics and Evolution" (C. L. Markert, ed.), pp. 433–448. Academic Press, New York.
Coates, P. M., Mestriner, M. A., and Hopkinson, D. A. (1975). A preliminary genetic interpretation of the esterase isozymes of human tissues. *Ann Hum. Genet.* 39, 1–20.
Crow, J. F., and Kimura, M. (1970). "An Introduction to Population Genetics Theory." Harper, New York.
de Ligny, W. (1969). Serological and biochemical studies of fish populations. *Oceanogr. Mar. Biol. Annu. Rev.* 7, 411–513.
de Ligny, W. (1972). Blood groups and biochemical polymorphisms in fish. *Eur. Conf. Anim. Blood Groups Biochem. Polymorphism, 12th*, pp. 55–65.
Donaldson, L. R., and Olson, P. (1955). Development of rainbow trout brood stock by selective breeding. *Trans. Am. Fish. Soc.* 15, 93–101.
Falconer, D. S. (1960). "Introduction to Quantitative Genetics." Ronald Press, New York.
Gall, G. A. E. (1972). Phenotypic and genetic components of body size and spawning performance. *In* "Progress in Fishery and Food Science" (R. W. Moore, ed), University of Washington Publications in Fisheries, New Series, Vol. 5, pp. 159–163. Univ. of Washington, Seattle.
Gall, G. A. E. (1974). Influence of size of eggs and age of female on hatchability and growth in rainbow trout. *Calif. Fish Game* 60, 26–36.
Gall, G. A. E. (1975). Genetics of reproduction in domesticated rainbow trout. *J. Anim. Sci.* 40, 19–28.
Gjedrem, T. (1975). Possibilities for genetic gain in salmonids. *Aquaculture* 6, 23–29.
Gjedrem, T. (1976). Possibilities for genetic improvement in salmonids. *J. Fish. Res. Board Can.* 33, 1094–1099.
Hedgecock, D., Shleser, R. A., and Nelson, K. (1976). Applications of biochemical genetics to aquaculture. *J. Fish. Res. Board Can.* 33, 1108–1119.
Hershberger, W. K., Brannon, E. L., Donaldson, L. R., Yokoyama, G. A., and Roley, S. E. (1976). Salmonid aquaculture studies: Selective breeding. *In* "1975 Research in Fisheries," Contrib. No. 444, p. 61. Annu. Rep., Coll. Fish., Univ. of Washington, Seattle.
Hjorth, P. (1971). Genetics of *Zoarces* populations. I. Three loci determining the phosphoglucomutase isoenzymes in brain tissue. *Hereditas* 69, 233–242.
Hochachka, P., and Somero, G. N. (1973). "Strategies of Biochemical Adaptation." Saunders, Philadelphia, Pennsylvania.
Huzyk, L., and Tsuyuki, H. (1974). Distribution of *LDH-B"* gene in resident and anadromous rainbow trout (*Salmo gairdneri*) from streams in British Columbia. *J. Fish. Res. Board Can.* 31, 106–108.

Jacquard, A. (1975). Inbreeding: One word, several meanings. *Theor. Popul. Biol.* 7, 338–363.

Johnson, A. G., Utter, F. M., and Hodgins, H. O. (1972). Electrophoretic investigation of the family Scorpaenidae. *U.S. Fish Wildl. Serv., Fish. Bull.* 70, 403–413.

Johnson, G. B. (1976). Hidden alleles at the α-glycerophosphate dehydrogenase locus in *Colias* butterflies. *Genetics* 83, 149–167.

Johnson, M. S. (1971). Adaptive lactate dehydrogenase variation in the crested blenny, *Anoplarchus*. *Heredity* 27, 205–226.

Kempthorne, O. (1957). "An Introduction to Genetic Statistics." Wiley, New York.

Kimura, M., and Maruyama, T. (1971). Pattern of neutral polymorphism in a geographically structured population. *Genet. Res.* 18, 125–131.

King, M. C., and Wilson, A. C. (1975). Evolution at two levels: Molecular similarities and biological differences between humans and chimpanzees. *Science* 188, 107–116.

Koehn, R. K. (1969). Esterase heterogeneity: Dynamics of polymorphism. *Science* 163, 943–944.

Kornfield, I. L., and Koehn, R. K. (1975). Genetic variation and speciation in New World cichlids. *Evolution* 29, 427–437.

Kosswig, C. (1973). The role of fish in research on genetics and evolution. *In* "Genetics and Mutagenesis of Fish" (J. H. Schroder, ed.), pp. 3–16. Springer-Verlag, Berlin and New York.

Krieger, H., Morton, N. W., Mi, M. P., Ezevedo, E., Freire-Maia, A., and Yasuda, N. (1965). Racial admixture in northeastern Brazil. *Ann. Hum. Genet.* 29, 113–125.

Kristiansson, A. C. (1975). Biochemical genetic variation among selected populations of chinook salmon (*Oncorhynchus tshwytscha*) in Oregon and Washington. M.S. Thesis, Oregon State Univ., Corvallis.

Lewontin, R. C. (1967). The principle of historicity in evolution. "Mathematical Challenges to the Neo-Darwinian Interpretation of Evolution," Wistar Institute Symposium Monograph No. 5, pp. 81–94. Wistar Inst., Philadelphia, Pennsylvania.

Lewontin, R. C. (1974). "The Genetic Basis of Evolutionary Change." Columbia Univ. Press, New York.

McKee, B. (1972). "Cascadia: The Geologic Evolution of the Pacific Northwest." McGraw-Hill, New York.

Markert, C. L., and Faulhaber, I. (1965). Lactate dehydrogenase isozyme patterns of fish. *J. Exp. Zool.* 159, 319–332.

May, B. P. (1975). Electrophoretic variation in the genus *Oncorhynchus:* The methodology, genetic basis, and practical applications to fisheries research and management. M.S. Thesis, Univ. of Washington, Seattle.

May, B., Utter, F. M., and Allendorf, F. W. (1975). Biochemical genetic variation in pink and chum salmon: Inheritance of intraspecies variation and apparent absence of interspecies introgression following massive hybridization of hatchery stocks. *J. Hered.* 66, 227–232.

Merritt, R. B. (1972). Geographic distribution and enzymatic properties of lactate dehydrogenase allozymes in the fathead minnow, *Pimephales promelas*. *Am. Nat.* 196, 173–184.

Millenbach, C. (1973). Genetic selection of steelhead trout for management purposes. *Int. Atl. Salmon J.* 4, 253–257.

Mitton, J. B., and Koehn, R. K. (1975). Genetic organization and adaptive response of allozymes to ecological variables in *Fundulus heteroclitus*. *Genetics* 79, 97–111.

Moav, R., and Wohlfarth, G. W. (1973). Carp breeding in Israel. *In* "Agricultural Genetics: Selected Topics" (R. Moav, ed.), pp. 293–318. Wiley, New York.

Nei, M. (1972). Genetic distance between populations. *Am. Nat.* **106**, 283–292.

Nei, M., and Roychoudhury, A. K. (1974). Sampling variances of heterozygosity and genetic distance. *Genetics* 76, 379–390.

Nyman, L. (1972). A new approach to the taxonomy of the "*Salvelinus alpinus* species complex." *Inst. Freshwater Res. Drottningholm, Rep.* No. 52, pp. 103–131.

Nyman, L. (1975). Allelic selection in a fish (*Gymnocephalus cernua* (L.)) subjected to hotwater effluents. *Inst. Freshwater Res. Drottningholm. Rep.* No. 54, pp. 75–82.

Ohno, S. (1970). "Evolution by Gene Duplication." Springer-Verlag, Berlin and New York.

Powell, J. (1975). Protein variation in natural populations of animals. *In* "Evolutionary Biology" (T. Dobzhansky, M. I. Hecht, and W. C. Steere, eds.), Vol. 8, pp. 79–119. Plenum, New York.

Purdom, C. E. (1972). "Genetics and Fish Farming," Lab. Leaflet (New Ser.) No. 25. Min. Agric. Fish. Food, Fish. Lab., Lowestoft, Suffolk, England.

Purdom, C. E. (1976). Genetic techniques in flatfish culture. *J. Fish. Res. Board Can.* 33, 1088–1093.

Prakash, S., Lewontin, R. C., and Hubby, J. L. (1969). A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal and isolated populations of *Drosophila pseudoobscura*. *Genetics* 61, 841–858.

Rasmuson, M. (1968). "Populationsgenetiska Synpunkter pa Laxodlingsverksamheten i Sverige," Rep. LFI Medd. 3/1968. Swed. Salmon Res. Inst., Stockholm.

Ridgway, G. J., Sherburne, S. W., and Lewis, R. D. (1970). Polymorphism in the esterases of Atlantic herring. *Trans. Am. Fish. Soc.* 99, 147–151.

Robertson, F. W. (1972). Value and limitations of research in protein polymorphism. *Eur. Conf. Anim. Blood Groups Biochem. Polymorphism, 12th* pp. 41–54.

Rogers, J. S. (1972). Measures of genetic similarity and genetic distance. Studies in Genetics VII, Univ. Texas Publ., 7213:145–153.

Schmidt, J. (1922). The breeding places of the eel. *Philos. Trans. R. Soc. London, Ser. B* **211**, 179–208.

Schmidt, J. (1925). The breeding places of the eel. *Smithson. Rep.* 1924, pp. 279–316.

Selander, R. K., and Johnson, W. E. (1973). Genetic variation among vertebrate species. *Annu. Rev. Ecol. Syst.* 4, 75–91.

Shaklee, J. B., Champion, M. J., and Whitt, G. (1974). Developmental genetics of teleosts: A biochemical analysis of lake chubsucker ontogeny. *Dev. Biol.* 38, 356–382.

Shaw, C. R. (1964). The use of genetic variation in the analysis of isozyme structure. *Brookhaven Symp. Biol.* No. 17, pp. 117–130.

Shaw, C. R., and Prasad, R. (1970). Starch gel electrophoresis of enzymes—A compilation of recipes. *Biochem. Genet.* 4, 297–320.

Simon, R. C. (1972). Gene frequency and the stock problem. *In* "The Stock Concept in Pacific Salmon" (R. C. Simon and P. A. Larkin, eds.), pp. 161–169. Inst. Fish., Univ. of British Columbia, Vancouver.

Singh, R. S., Hubby, J. L., and Lewontin, R. C. (1974). Molecular heterosis for heat-sensitive enzyme alleles. *Proc. Natl. Acad. Sci. U.S.A.* 71, 1808–1810.

Sneed, K. E., Pillay, S. R., and Rabanal, H. R., eds. (1971). "Seminar/Study Tour in the U.S.S.R. on Genetic Selection and Hybridization of Cultivated Fishes, 19 April—29 May, 1968, Lectures," UN Dev. Programme No. TA 2926. FAO *U.N.*, Rome.

Soule, M., Yang, S. Y., Weiler, M. G., and Gorman, G. C. (1973). Island lizards: The genetic–phenetic variation correlation. *Nature (London)* 242, 190–192.

Turner, B. J. (1974). Genetic divergence of Death Valley pupfish species: Biochemical versus morphological evidence. *Evolution* 28, 281–294.

Utter, F. M., Ames, W. E., and Hodgins, H. O. (1970). Transferrin polymorphism in coho salmon (*Oncorhynchus kisutch*). *J. Fish. Res. Board Can.* 27, 2371–2373.

Utter, F. M., Allendorf, F. W., and Hodgins, H. O. (1973). Genetic variability and relationships in Pacific salmon and related trout based on protein variations. *Syst. Zool.* 22, 257–270.

Utter, F. M., Hodgins, H. O., and Allendorf, F. W. (1974). Biochemical genetic studies of fishes: Potentialities and limitations. *In* "Biochemical and Biophysical Perspectives in Marine Biology" (D. C. Malins and J. R. Sargent, eds.), Vol. 1, pp. 213–238. Academic Press, New York.

Williams, G. C., Koehn, R. K., and Mitton, J. B. (1973). Genetic differentiation without isolation in the American eel, *Anguilla rostrata*. *Evolution* 27, 192–204.

Wilmot, R. L. (1974). A genetic study of the red-band trout (*Salmo* sp.). Ph.D. Thesis, Oregon State Univ., Corvallis.

Withler, I. L. (1966). Variability in life history characteristics of steelhead trout (*Salmo gairdneri*) along the Pacific coast of North America. *J. Fish Res. Board Can.* 23, 365–393.

Wolf, U., Engel, W., and Faust, J. (1970). Zum Mechanismus der Diploidisierung in der Wirbeltierevolution: Koexistenz von tetrasomen und disomen Genloci der Isocitrat-Dehydrogenases bei der Regenbogenforelle (*Salmo irideus*). *Humangenetik* 9, 150–156.

Wright, J. E., Heckman, J. R., and Atherton, L. M. (1975). Genetic and developmental analyses of LDH isozymes in trout. *In* "Isozymes," Vol. 3, "Developmental Biology" (C. L. Markert, ed.), pp. 375–401. Academic Press, New York.

Yardley, D., Avise, J. C., Gibbons, J. W., and Smith, M. H. (1974). Biochemical genetics of sunfish. III. Genetic subdivision of fish populations inhabiting heated waters. *Therm. Ecol., Proc. Symp., Augusta, Ga., USAEC, 1973* pp. 255–263.